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4297

Properties of Unmyelinated Fibers of Nerve.*

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Previous reports on the physiology of nerve by Erlanger, Gasser and Bishop[1] have dealt with the properties of the myelinated fibers. In this paper are presented data on the properties of fibers characteristic of the involuntary nervous system especially the unmyelinated ones. The analyses have been made by means of the cathode ray oscillograph and the use of amplification greater than was usually considered necessary for the study of the myelinated fibers.

The unmyelinated fibers can be identified by a conduction rate slower than that of the ordinary myelinated fibers of peripheral nerves (1.5 to 0.2 M. per sec. in the turtle). These rates are comparable to the rates reported by Chauchard, A. et B.,[2] as determined from the effect of stimulation at different levels along the course of nerves containing unmyelinated fibers. A second characteristic of these fibers is their high threshold. An induction shock at least 10 to 100 times that of the most irritable fibers in the same nerve is required for a threshold response in the turtle, in the cat 50 to 100 times.

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* This preliminary report on unmyelinated nerve fibers is published as one phase of the more general problem of a comparative study of the differences between myelinated and unmyelinated axons, being carried out by the author and Dr. George H. Bishop.

[1] Erlanger, Joseph, Bishop, George H., Gasser, H. S., *Am. J. Physiol.*, 1926, lxxviii, 537.

[2] Chauchard, A. et B., *Compt. Rend. Soc. de Biol.*, 1925, xcv, 279 and 370.

A more suitable method of stimulating these fibers is by the galvanic current. The nerve is arranged in a bridge balanced for resistance and capacity as described by Bishop.[3] Relative thresholds can then be measured either by change in duration or by change in intensity of stimulating current. On the basis of either threshold or conduction rate the fibers, if myelinated, would have to be not more than 0.1μ in diameter in order to fit the size conduction rate ratio of Gasser and Erlanger[4] for the myelinated fibers. Myelinated fibers of this diameter are not found in the nerves under consideration.

Using the galvanic current in the manner indicated above, the chronaxie of these fibers can be measured. As read from the complete curves of time against intensity their chronaxies range from approximately 3.5 to 6 sigma as compared with 0.3 sigma for the myelinated fibers of the frog or turtle first processes.

In recording the action potential duration at or near the stimulated point, in order to obtain the form of the potential in the individual axon, considerable difficulty was met, due to the strong stimulating current required. Since the threshold of these fibers is 10 to 100 times that of the first myelinated fibers stimulated and since the sensitivity required for recording them is at least 10 times as great, the record of the stimulating current (escape) will be at least 100 to 1000 times that of the currents necessary for stimulating the most irritable fibers. Very careful balancing of the bridge circuit is necessary both to decrease the direct record of the shock and to eliminate distortion due to the capacity of the stimulating circuit itself. In this we have not been wholly successful to date and our results are presented as approximate ones. There appears to be no question, however, that the duration of the potential at the stimulated point is considerably longer than that of the ordinary myelinated axon. This is indicated by different methods, (1) direct recording in a balance bridge circuit, (2) stimulation by 2 shocks, the second applied during the refractory phase of the first process arising from the myelinated fibers, and (3) extrapolation back to the stimulated point. Of the duration of the conducted action potential all indicate a longer duration of the axon action potential of fibers possessing the properties of the unmyelinated ones than that arising from the ordinary myelinated fibers. The ratio of their rising phases appears to be between 5 and 10 to 1.

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[3] Bishop, George H., *Am. J. Physiol.*, 1927, lxxii, 462.

[4] Gasser, H. S., and Erlanger, Joseph, *Am. J. Physiol.*, 1927, lxxx, 522.

The absolutely and relatively refractory phases of these fibers have been determined by plotting the curve of return of irritability after a maximal first stimulus. The potentials were recorded after conduction to allow separation out from the earlier waves (Gasser and Erlanger).[5] Condenser charges were employed usually up to 160 volts and from .01 to .1 microfarad capacity. These condensers were connected through 2 contacts of the circuit breaker and charged in series with the bridge containing the nerve, the whole being balanced for capacity and resistance. To prevent the first condenser shunting the second, the circuit breaker was made to open the first circuit before the second was closed, the condenser charging through the nerve circuit during this interval. Both repeated and single pairs of stimuli were employed and it was found that stimuli applied more frequently than at the rate of 1 per second resulted in a prolongation of the absolutely refractory phase. The nerve was supplied with oxygen at room temperature. Preliminary experiments on the effect of anoxemia on these fibers and the knowledge that in the ordinary myelinated fibers, failure of response occurs when the normal absolutely refractory period of less than one sigma has increased only 100 to 300%, both indicate that the long absolutely refractory period found for these fibers is not the result of depression by anoxemia. They have an absolutely refractory period of 4 to 6 sigma when the action potential is still maximal, as indicated by the results of single stimuli.

These measurements of the properties of the unmyelinated fibers are being used to differentiate between types of fibers found in the same nerve.

4298

The Direct Estimation of True Blood Sugar.

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Blood sugar values obtained by Somogyi's[1] fermentation technique very probably represent the *true sugar* of blood. This procedure, while the standard for comparison, is somewhat laborious, requiring 2 determinations for one result. It has been found that blood filtrates prepared by precipitation with acid mercuric sulphate followed by neutralization with barium carbonate and removal of the mercury with zinc give true sugar values as judged by the fermentation procedure. Barium carbonate possesses an advantage as a neutralizing agent in that it not only removes the sulphate from solution but automatically adjusts the pH to a uniformly definite value at which reducing non-sugars are efficiently precipitated by the mercury.

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[5] Gasser, H. S., and Erlanger, Joseph, *Am. J. Physiol.*, 1925, lxxiii, 613.

[1] Somogyi, M., *J. Biol. Chem.*, 1927, lxxv, 33.

Method: 5 cc. of blood (1 vol.) are laked in 50 cc. of water (10 vol.) and 5 cc. (1 vol.) of acid mercuric sulphate (30% $HgSO_4$ in 10% H_2SO_4) added, with shaking, from a burette. The flask is stoppered and thoroughly shaken to break up the gel. 9-10 gm. of dry precipitated barium carbonate are added, the flask is rotated for a few seconds until most of the CO_2 has escaped, and then stoppered and shaken. Carbon dioxide is released until no further pressure develops in the flask and the mixture reacts neutral to litmus paper. If neutralization does not take place promptly, add 1-2 gm. additional barium carbonate. The mixture is poured upon a rapid filter. The filtrate (about 22 cc.) is treated with one drop of saturated sodium sulphate (to remove traces of barium) a pinch of zinc dust, and the flask shaken for a second or two. Zinc is filtered off and the sugar determined by the Shaffer-Hartmann method using an unpublished modification of the reagent by Dr. Somogyi. It has been found better than his published modification and is of the composition:

	gm. per liter
Sodium carbonate, anhydrous	20
Sodium bicarbonate	25
Rochelle salt	25
Copper sulphate, cryst.	7
Potassium oxalate	5
Potassium iodide	10
Potassium iodate	0.8

The time required for the preparation of filtrates is about the same as by the method of Folin-Wu. The mixture filters quickly and rapid filters may be used. Upon standing, a slight precipitate of zinc carbonate separates from the filtrates which, however, in no way interferes with the sugar determination. The filtrates contain a minimum of electrolytes because all the precipitating agent is removed by the barium carbonate and zinc. Nitrogen determinations show the filtrates of normal blood to contain no detectable N. The technique is useful in preparing filtrates of tissue extracts, etc. Further work is in progress and a more detailed report will be published elsewhere.

4299

A Method for the Preparation of Blood Filtrates for Analysis.

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From the Laboratory of the Jewish Hospital of St. Louis.

The use of zinc salts for the precipitation of blood proteins furnishes a method of preparing filtrates in which fermentable sugar (glucose) is the only substance to reduce alkaline copper solutions. The reducing substances other than sugar—responsible for the "residual reduction" of fermented blood filtrates—are precipitated along with the proteins so that sugar determinations in zinc filtrates give true blood sugar values.

In a simple method of deproteinization we employ the following 2 reagents:

Reagent I. 12.5 g. of zinc sulphate ($ZnSo_47H_2O$) are dissolved in water, 125 cc. of 0.25 normal sulfuric acid are added, and made up with water to one liter.

Reagent II is a 0.75 normal solution of sodium hydroxide.

The 2 reagents have to be in such a definite relation that when Reagent I is titrated against Reagent II in the presence of phenol phtalein, 25 cc. of I should require 3.35 to 3.40 of II to produce a permanent pink color.

Procedure: In order to prepare a 1 :10 filtrate, lake one volume of blood in 8 volumes of Reagent I, add one volume of Reagent II, stopper tightly, shake well, filter after a few minutes through a dry filter paper. Centrifuge before filtration if a maximum yield of filtrate is wanted.

For the preparation of filtrates of concentrations other than 1 :10, we use a somewhat different technique and pair of reagents. Reagent *A* is a 19.5% zinc sulphate solution, Reagent *B* a normal sodium hydroxide. One volume of blood requires one half a volume of each of these reagents for deproteinization. If, for instance, a 1 :5 filtrate is desired, lake one volume of blood in 3 volumes of water, introduce one half a volume of Reagent *A*, mix, then add one half a volume of Reagent *B*, stopper tightly, shake well, filter after a few minutes. The 2 reagents must be so adjusted that 10 cc. of *A* should require 11 cc. of *B* to produce a permanent pink color in the presence of phenol phtalein.

Filtrates prepared by either of the 2 procedures yield sugar values (Shaffer-Hartmann method, modified reagent) substantially identical with the true sugar values obtained from tungstic acid filtrates as the difference of apparent sugar (total reduction) and reducing non-sugars (residual reduction). The zinc filtrates are free from uric acid, glutathione and ergothioneine, do not consume iodine in the presence of acid, and possess no reducing power after treatment with washed yeast.[1] Pure glucose, added to fermented zinc filtrates, is quantitatively recovered. These facts support the view, advanced by the writer in a previous paper[2] that glutathione and ergothioneine represent the bulk of the non-sugar reducing substances in blood.

The non-protein nitrogen values are lower in zinc filtrates than in tungstic acid filtrates. The difference is 8 to 15 mg. per 100 cc. of blood, a figure closely corresponding to the aggregate amount of glutathione, ergothioneine and uric acid, the substances precipitated along with the proteins. The non-protein nitrogen values of zinc filtrates seem to represent urea with creatine and creatinine. Experiments concerning the partition of non-protein nitrogen of zinc filtrates are in progress in this laboratory.

4300

Growth of Rats on Diets Containing Sodium Benzoate.

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The increase in weight of young male white rats during a 40 day experimental period on diets containing sodium benzoate has been determined. The food consumption was regulated so that all of the rats received equal quantities of food of equivalent calorific value. The increase in weight of rats receiving the basal diet plus 1.5, 2.0 or 2.5% sodium benzoate was practically the same as that of rats receiving the basal diet. On the diet containing 3% sodium benzoate one-third of the rats died and the growth of the remainder was distinctly less than that of the control rats even though the food consumption was the same. The addition of glycine or of gelatin to the diet containing 3% sodium benzoate resulted in growth equal to that of rats on the basal diet. These results, based upon the consumption of equal amounts of food of the same calorific value, are in agreement with our earlier experiments in which similar diets were fed *ad libitum.*

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[1] Somogyi, *J. Biol. Chem.*, 1928, xxviii, 9.

[2] Somogyi, *J. Biol. Chem.*, 1927, lxxv, 33.

4301

Apparatus for the Quantitative Testing of Air and Bone Transmitted Speech.

A. G. POHLMAN.

From the Department of Anatomy, St. Louis University.

The usual test for the relative acuity of hearing air and bone transmitted sound is made with a series of tuning forks—the air vibrations coming from the prongs and the bone vibrations through application of the stem to the head of the examined individual. It is well known that the greatest disability of the deafened individual is found in his inability to hear conversations. The sounds from the fork are relatively pure tones while the voice sounds are complicated combinations of tones. For this reason the fork tests are a poor criterion of the sensitivity for speech. An air telephone with its large diaphragm and efficient production of air vibrations of large amplitude and little force necessarily shows an entirely different type of response to that elicited through a bone telephone which develops small amplitudes of considerable force. A quantitative method of producing voice sounds has been developed by the Western Electric Co. in the phonograph audiometer with its electromagnetic pick-up. This instrument may also be employed with a bone telephone provided the energy is stepped up with a 2 stage audio-amplifier. It is also possible to test both the air and the bone acuity with the same receiver under the same mechanical conditions. This quite eliminates the physical peculiarities of the receiver itself and makes a direct comparison possible. The air acuity is determined by holding the receiver by its mushroom stem against a resonant surface and the person examined writes the numbers heard which become progressively fainter and fainter. Next the record is changed and the bone telephone is held against the forehead in a similar manner. Again the numbers are written by the observer. If it is thought desirable to test the enhancement to bone acuity which is brought about by occlusion of the external canals, the ears are closed with plastocene and the test repeated the third time.

The advantage of employing a single receiver to accomplish both purposes is obvious. Further the test is sufficiently interesting to help rivet the attention on the problem; eliminates the personal equation of the examiner; and is both rapid and satisfactory. A demonstration of the apparatus was made to prove the surprisingly good quality of reproduction both through air and through bone vibrations.

4302

Effect of Amylase on Formation of Hexose-phosphate by Muscle Extract.

ETHEL RONZONI. (Introduced by P. A. Shaffer.)

From the Department of Internal Medicine, Washington University, St. Louis.

The mechanism by which the pancreatic factor[1] inhibits the formation of hexose-phosphate[2] and therefore glycolysis in muscle extract has been shown by Case and McCullagh[3] to be a direct action on the starch or glycogen used as the source of carbohydrate. Hydrolysis of these carbohydrates before the esterification of their mono-saccharid derivatives with phosphate seems a necessary assumption. Complete inhibition is difficult to explain since always there remains a residue of carbohydrate with but slight reducing value. We are, however, able to confirm these observations and find also that muscle amylase added in sufficient quantities produces marked inhibition of glycolysis.

We report here a study of the action of pancreatic diastase on the formation of hexose-phosphate by muscle extract, undertaken with a view to establishing a possible rôle of amylase in muscle extract glycolysis. If hydrolysis of starch or glycogen is a necessary step before its esterification with phosphate, it should be possible to increase the rate of esterification by accelerating the hydrolysis of the carbohydrate, unless muscle extract contains an optimum amount of amylase for this purpose. Muscle extract prepared by extraction with salt solution for one-half hour has an almost optimum amylolytic activity and the addition of small quantities of amylase has no effect or slightly inhibits the formation of hexose phosphate. However, muscle extracts prepared by brief extraction with water have a lower amylolytic activity and on these extracts the acceleration in the rate of formation of hexose-phosphate by added amylase is shown in the following chart. To the water extract of muscle Na_2HPO_4 was added to give a concentration of 200 mg. P. per 100 cc. The concentration of $NaHCO_2$ was .025 M. and NaF .02 M. To equal quantities of this extract equal amounts of water or amylase solution were added. After the addition of starch to give a concentration of 500 mg. %, the rate of formation of hexose-phosphate in 2 cc. of such an extract was followed by manometric determination of CO_2 liberated from $NaHCO_3$ by the formation of the more acid hexose-phosphate.[4] Phosphate changes were determined at the end of the reaction period. This curve would be influenced by small accumulation of acids other than lactic, since the latter was determined chemically and found not to increase.

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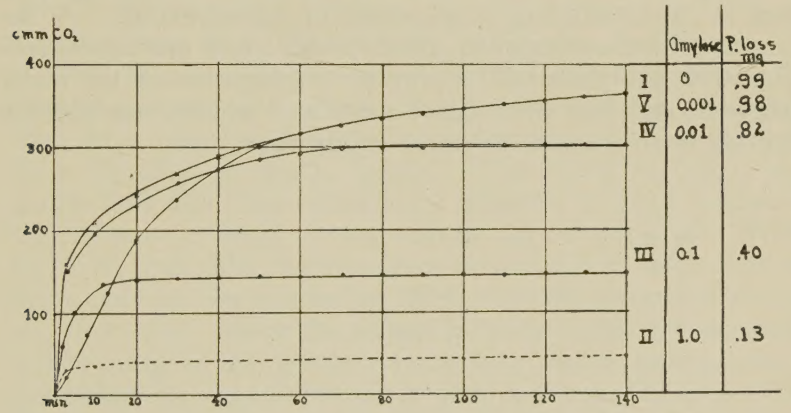
[1] Winfield, G., and Hopkins, F. G., *J. Physiol.*, 1915, l, 5.

[2] Ronzoni, E., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 178.

[3] Case, E. M., and McCullagh, D. R., *Biochem. J.*, 1928, xxii, 1060.



The chart shows that even when marked inhibition in the total production of CO_2 occurs the rate of formation is accelerated. With smaller amounts of amylase although the total amount formed in 140 minutes is not increased the initial rate of its production is accelerated.

Changes in free reducing substance and in phosphates were followed chemically in samples of the same extract. These cannot be compared directly with manometric determinations since they were kept at room temperature about 3° C. below that of the water bath and were not shaken. The following Table I shows the increase in reducing substance expressed as glucose in mg. % formed from a total carbohydrate concentration of 500 mg. %.

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[4] Meyerhof, O., *Biochem. Z.*, 1926, clxxviii, 427.

TABLE I.

	I	II	III	IV	V	
Time Min.	0	1	.1	.01	.001	Amylase added
10	0	42	4	0	0	increase in reducing substance Mg. %
15	0	60	6	6	0	
30	4	94	20	8	6	
140	10	112	50	16	14	
	Phosphate decrease (mg. 10 min.) per 10 cc. muscle extract.					
10	1.2	.55	1.3	2.11	2.20	

From this and other experiments of the same type, it is evident that muscle extract may be prepared with less than the optimum concentration of amylase, and that in such cases the rate of formation of hexose-phosphate may be accelerated by the addition of solutions containing amylase, thus establishing the fact that hydrolysis of starch is a necessary step in glycolysis of muscle extract. A further search for the changes in carbohydrates which prevents esterification with phosphate will require an investigation of the earlier changes taking place when massive doses of amylase are added to relatively small amounts of starch or glycogen.

New York Meeting.

New York Academy of Medicine, February 20, 1929.

4303

Comparison of Resistance Changes in Collodion Membranes and Cells.

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From the Laboratories of The Rockefeller Institute for Medical Research.

Most conductors, both metallic and electrolytic, follow Ohm's law, the current being proportional to the applied potential, and the resistance a constant (with exceptions in the case of temperature rise or dielectric breakdown). In the large cells of *Valonia* and *Nitella,* however, the resistance becomes higher with increase of potential from 25 to 150 millivolts, under some conditions.[1]

This discovery led to a search for a non-living model of this phenomenon and such has been found in dry collodion films, thinner than those used by Michaelis,[2] and Northrop;[3] thicker membranes show the effect in smaller degree. When direct current is passed across such thin films separating 2 solutions of good conductivity, the resistance is found to vary with the applied potential. With sea water on both sides, the resistance is a minimum below 100 millivolts, and may rise as much as 100% when the potential is increased to 3 or 4 volts. Above this voltage irregular results are obtained.

The course of the resistance rise is very regular and reproducible, resembling the exponential curve of a condenser charging-current. There is, however, no E.M.F. of polarization, detected on breaking the current, which could account for more than a few per cent of the increase of resistance. The lower value is regained apparently as soon as the return to a lower measuring potential is made; although some effect persists, since the second and third applications of high potential in quick succession cause more rapid rise of resistance than the first.

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[1] Blinks, L. R., *Am. J. Physiol.,* 1928, lxxxv, 351.

[2] Green, A. A., Weech, A. A., and Michaelis, L., *J. Gen. Physiol.,* 1928-29, xii, 473.

[3] Northrop, J. H., *J. Gen. Physiol.,* 1928-29, xii, 435.

When the membrane separates 2 solutions of the same concentration but of different conductivity, such as 0.5 N KCl and HCl, the direction of the current is found to influence the resistance change. Thus when hydrogen ion is moved across the membrane, the resistance falls slightly with increasing potential, while when potassium ions are caused to pass by reversing the current, the resistance rises as much as 100% in changing from 0.1 to 3.0 volts. Somewhat similar differences are found when the membrane separates 2 different concentrations of the same solution.

There seems also to be an effect of the membrane itself when the same solution is used on both sides, causing a lower resistance in one direction than in the other. This may be due to the method of drying one surface in contact with glass.

The cause of the effect is perhaps to be sought in the changes of mobility which ions undergo when in such membranes (*cf.* Northrop and Michaelis). Probably also a concentration gradient is quickly established within the membrane which is disturbed by the application of a potential gradient, causing a new distribution of the electrolyte, even for short distances into the surrounding solution. Movement of water due to electro-endosmosis seems unlikely in view of the concentrations of salt used (0.1 to 1.0 m).

Photographic records of current changes in the measuring bridge show marked resemblances to those taken with *Valonia* and *Nitella* and the causes of the phenomenon may be similar in these cases. On the other hand, variations of resistance in human skin are probably more complex.[4]

4304

The Inverse Relation Between Inorganic and Lipoid Phosphorus in Blood of Rabbits.

ALVIN R. HARNES. (Introduced by Wade H. Brown.)
From the Laboratories of The Rockefeller Institute for Medical Research.

In a recent paper,[1] attention was called to the fact that, among rabbits living out of doors, the inorganic phosphorus of serum and the lipoid phosphorus of whole blood show wide variations with a high negative coefficient of correlation, –0.794 ± .09. The curve for inorganic phosphorus over a period of 12 months was almost a mirror image of the curve for lipoid phosphorus (Fig. 1).

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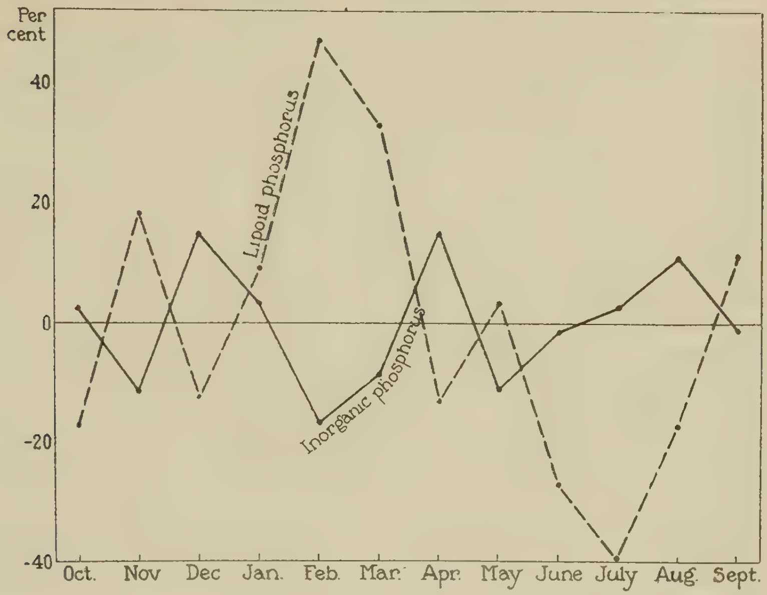
[4] Gildemeister, M., *Handb. norm. u. path. Physiol.*, 1928, viii, pt. 2, 657.

[1] Harnes, A. R., *J. Exp. Med.*, 1928, xlviii, 549.



Additional experiments were performed for the purpose of testing the influence of light on this relation. Inorganic phosphorus of serum and lipoid phosphorus of whole blood were studied on 4 groups of rabbits, one of which was kept in the open laboratory, another was exposed continually to Cooper Hewitt light, and the 2 other groups were kept in the dark; one of these was irradiated for one hour daily with a quartz-mercury arc lamp at a distance of 4.5 meters. The observations covered a period of 4 months.

The irradiated animals showed a high inorganic and a low lipoid phosphorus while the 3 other groups showed a lower inorganic and a higher lipoid phosphorus. But, the variations were such that, in all cases, the sum of the mean values for inorganic and lipoid phosphorus were nearly constant.

The correlation coefficients were negative in all cases but were of a low order for all except the ultra-violet group.

Of the 6 groups of animals in which the relation between inorganic and lipoid phosphorus has been studied, all have shown evidence of an inverse relation with a tendency to maintain a constant sum of the 2 forms. In only 2 cases, however, has the relation been sufficiently close to give a correlation coefficient of a high order; animals exposed to unfiltered sunlight and those irradiated with the quartz-mercury arc gave high coefficients, while with others the correlation was low.

That an inverse relation exists in man is evident from the results obtained by McKellips, De Young and Bloor,[2] who found in the blood of normal infants a low lipoid phosphorus and a high inorganic phosphorus; in the blood of adults, a high lipoid phosphorus and a low inorganic phosphorus. McCluskey[3] found the same inverse relationship in tuberculous patients.

It is also of interest to note that Zucker and Gutman[4] report that in experimental rickets the inorganic phosphorus is decreased while the organic phosphorus is practically constant.

These results show that in the rabbit there is an intimate relation between the inorganic and lipoid phosphorus of the blood, and that the maintenance of this relation is influenced in a very striking manner by ultra-violet light. The conditions found suggest a reversible reaction which is subject to the influence of light. There is the further suggestion that the mechanism concerned in the maintenance of a normal equilibrium between inorganic and lipoid phosphorus in the blood may prove to be an important factor in diseases such as rickets, as it appears that the low inorganic phosphorus in the blood is not necessarily associated with a decrease in other forms of phosphorus or is not due to a phosphorus starvation.

4305

The Experimental Transmission of Leukemia in Mice.

MAURICE N. RICHTER AND E. C. MAC DOWELL.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, and the Department of Genetics, Carnegie Institution of Washington.

The only report in the literature of the successful transmission of leukemia in mammals, is that of Snijders,[1] who succeeded in transmitting the disease by the inoculation of emulsions of tissues from spontaneously affected guinea pigs into normal animals of the same species. Unsuccessful attempts to transmit leukemia in mice were made by Haaland[2] and Tyzzer.[3]

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[2] McKellips, De Young, and Bloor, *J. Biol. Chem.*, 1921, xlvii, 53.

[3] McCluskey, K. Lucille, *J. Lab. and Clin. Med.*, 1925, x, 466.

[4] Zucker, T. F., and Gutman, M., PROC. SOC. EXP. BIOL. AND MED., 1922, xx, 372.

[1] Snijders, E. P., *Nederl Tijdschr. v. Geneesk.*, 1926, lxx, 1256. Transmissible leukemia in the fowl was first described by Ellermann and Bang in 1908. For a comprehensive review of this and other experimental work on leukemia, with bibliography, see Opie, E. L., *Medicine*, 1928, vii, 31.

In a strain of mice designated hereafter as C58, which has been inbred by brother-sister matings since 1921, it was observed that a considerable number of those that lived more than 8 months had enlargements of their spleens and lymph nodes. Microscopic examination by Dr. Alwin M. Pappenheimer in 1926 disclosed that these enlargements were due to lymphatic leukemia.

The present cooperative investigation was undertaken early in 1928, at which time the number of matings in this strain was increased.

Of 42 mice in the 16th and 17th generations that lived more than 8 months,[4] 29 have had splenomegaly. Of these, 19 have been diagnosed as leukemia by microscopic examination, 2 presented the gross anatomical lesions of leukemia and are awaiting microscopic diagnosis, 2 had the gross lesions of leukemia but are not available for microscopic study, and 6 are still alive. Four mice died or were killed at about 10 months of age, but were not leukemic. Nine mice are still living and at present do not have leukemic symptoms.

Tumors other than those occurring as part of the leukemic syndrome are uncommon in this strain.

Saline emulsions of spleens from 6 mice spontaneously affected with lymphatic leukemia were inoculated intraperitoneally and subcutaneously into non-leukemic mice from the same and also from other strains. The non-leukemic mice were young animals of both sexes, from 1 to 2 months old. Following inoculation there appeared, in each of the 6 experiments, animals with enlarged lymph nodes and spleens, and with masses or nodules of lymphoid tissue in the abdominal cavity, sometimes forming large tumor-like growths in the mesentery, around the kidneys, or along the genital tract. Subcutaneous nodules, enlargement of superficial lymph nodes, mediastinal growths, and fluid in the peritoneal or pleural cavities were also frequently observed.

Microscopically, these growths consist of lymphoid cells in a delicate stroma. Many cells are in mitosis and there is infiltration of surrounding structures. Infiltration of the liver, pancreas, or lung is common.

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[2] Haaland, M., 1911, *Fourth Sc. Rept. Imperial Cancer Res. Fund.*

[3] Tyzzer, E. E., *J. Med. Res.*, 1907, xvii, 137.

[4] Slye reports that leukemic cases under 8 months of age have not appeared in her stock of mice. Slye, M., *J. Med. Res.*, 1915, xxxii, 159.

In some of the inoculated mice there was a striking increase in the number of circulating white corpuscles. The highest count recorded in the experiments was 262,000 per cm., about 3 weeks after inoculation. The increase in cells was due to an absolute and relative increase in the number of lymphocytes, many of which were immature. In some instances, small numbers of abnormal cells were observed in the blood even when the total white count was normal.

Similar changes have resulted from the inoculation of emulsions of these experimentally produced lymphoid enlargements, and the condition has been successfully transmitted through several experimental passages. One line has now reached the sixth transfer.

Of 110 mice of strain C 58 that lived more than 10 days after inoculation, 36 are still alive, and 74 have died or been killed. Of the latter, transmission was successful in 56, unsuccessful in 10. Diagnosis is not yet complete in 8.

Fourteen animals of other strains which lived more than 4 days after inoculation with the same material, have had neither lymphatic enlargements nor leukemic blood pictures. Thirteen are still alive. One died but presented no lesions resembling leukemia.

4306

Gram Structure of Cocci.

JOHN W. CHURCHMAN.

From the Laboratory of Experimental Therapeutics, Cornell Medical College.

It has been shown[1] that when *B. anthracis* is exposed, in aqueous suspension, to certain dyes (Gentian Violet, Acriviolet, etc.) its Gram reaction is reversed; and that this reversal is accompanied by diminution in size and loss of weight of the bacterial bodies, together with increase in weight of the supernatant fluid. The existence of an outer Gram-positive cortex and inner Gram-negative medulla seemed thus to be proven. Gram-positive cocci could not be similarly changed by exposure to these dyes and it was, therefore, assumed that the Gram reaction of cocci might rest on a different mechanism from that of *B. anthracis*.

Experimental study running over the last 18 months, has shown, however, that exactly the same result as that produced in *B. anthracis* by exposure to certain dyes, may be produced in certain of the cocci by exposure to a temperature of 52° Centigrade for a period of 10 hours or so. (Figs. 1 and 2.) Aqueous suspensions of many strains of *Staphylococcus aureus* when treated in this way become entirely Gram-negative. They also diminish in size, but the organisms are too small to measure accurately with a filar micrometer so that the amount of loss in diameter cannot be exactly stated. When centrifugated in Hopkins tubes, the organisms which have been exposed to 52° occupy less than half the space taken by the controls. They also lose about 50% in weight. This loss in weight is accompanied by a corresponding, though not an exactly identical, increase in the weight of the supernatant fluid.

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[1] Churchman, J. W., *J. Exp. Med.*, 1927, xlvi, 1007.

The reaction does not occur in all strains of cocci. For example, the Gram reaction of *M. freudenreichii* is entirely unaffected by exposure to 52° C. On the other hand, the *Staphylococcus aureus* with which most of the experiments here reported were done—a virulent and typical organism isolated from a human being—behaved with great constancy in this respect after certain of the puzzling factors of inconstancy met with in the early experiments had been determined and taken into account. These factors were, strength of bacterial suspension, amount of suspension, and possibly the shape of the vessel in which the suspension was placed. A large series of experiments showed that constant results could be obtained with this and a number of other strains if, (a) not too strong a bacterial suspension were used, and (b) not too large an amount of bacterial suspension. Thus, when .05 cc. of suspension A* of *Staphylococcus aureus* were placed in a pipette and exposed over night in an incubator at 52° C., absolutely constant results were obtained. Complete or nearly complete reversal of Gram reaction, with marked diminution in size, always occurred. On the other hand, if 50 cc. of suspension B† were placed in a 100 cc. Florence flask and similarly exposed to 52° C., reversal of Gram reaction and loss in size and weight frequently failed to occur. The reversal of Gram reaction by exposure to 52° C. could also with constancy be entirely prevented by the addition to the bacterial suspension of 2 parts of Sodium Carbonate. (Fig. 4.)

That reversal of Gram reaction is not simply a death phenomenon is proven by the fact that if *Staphylococcus aureus* be killed in other ways—for example, by boiling—reversal is not produced; and by the further fact that though exposure of *M. freudenreichii* to 52° C. kills this organism, it does not affect its Gram behavior.

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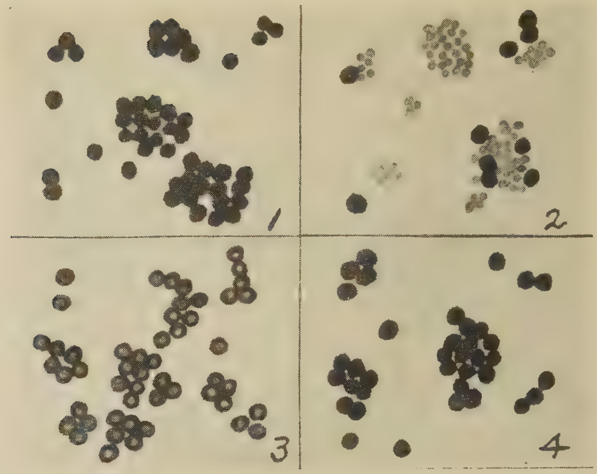
* Suspension A contained about 325,000,000 organisms per cc.

† Suspension B contained 20,000,000,000 organisms per cc.



1. Gram stain (Burke's modification) *Staph. aureus.* Control
2. Same stain after organisms have been exposed to 52° over night. Majority of organisms are now Gram negative and greatly reduced in size.
3. Effect of a pink center and purple periphery (similar to Gutstein picture) when Staphylococci are stained with methyl-violet and examined with bright illumination.
4. Gram stain of *Staph. aureus* which has been exposed, in sodium carbonate suspension, to 52°. No reversal or change of size (*Cf.* c Fig. 2).

The gain in weight which occurs in the fluid portion of bacterial suspension in which reversal of Gram reaction has been produced, is accompanied by the appearance of a positive ninhydrin test, and (if large amounts of suspension be used and the supernatant condensed by evaporation) of a positive Millon. It is hard to escape the conclusion from all the evidence that, when Gram reversal is brought about by exposure of cocci to 52° C., substance actually leaves the surface of the organisms whose Gram reaction has been reversed and passes into the fluid in which they are suspended.

By these studies, 3 grades of Gram positivity have thus been established: 1. *M. freudenreichii* remains positive after exposure to 52° and after exposure to the dyes mentioned. 2. Many cocci which show no change when exposed to the dyes mentioned are reversed in Gram reaction by exposure to 52°. 3. *B. anthracis* and many other spore-bearing aerobes become Gram negative either when exposed to the dyes mentioned or subjected to 52° C.

That the evidence thus advanced for the existence of an outer Gram positive cortex and an inner Gram-positive medulla is quite different in character from that advanced by Gutstein[2] for an ectoplasm and endoplasm ought to be sufficiently clear. Gutstein's evidence was tinctorial in character. Whatever interpretation be put upon his findings, it is certain that a picture identical with that obtained by Gutstein's staining method can be obtained without the use of any pink dye at all. If staphylococci be lightly stained with methyl-violet alone, the excess of dye taken off with alcohol applied for a couple of seconds and the smear examined with bright illumination, the organisms appear to have a pink center and a purple periphery exactly as described by Gutstein. (Fig. 3.) The same picture can be obtained by examining anthrax bacilli in a hanging drop to which a small amount of methyl-violet has been added. The axes of the organisms appear pink—their peripheries purple. This can hardly be interpreted as a tinctorial demonstration of ectoplasm and endoplasm; it is due entirely to the effect of bright light on the purple color.

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[2] Gutstein, N., *Centralbl. f. Bakt.*, 1924, i, 223.

In the experiments here recorded, the evidence offered for the existence of a Gram-positive bacterial cortex consists in the demonstration that this cortex can be removed by chemical and physical means and that its removal can be proven by a loss of weight in the bacterial bodies and by a gain in weight in the fluid in which they are suspended. It is also definitely shown that, although the cortex has been Gram-positive, the medulla which remains after its removal is Gram-negative.

4307

Characteristic Progressive Changes in the Hypertension Roentgenogram and Electrocardiogram.

ARTHUR M. MASTER.

One hundred fifty-two private cases of hypertension were followed for periods of between 3 and 9 years. Of these, 90% were patients with essential hypertension. Teleoroentgenograms, electrocardiograms and blood pressure readings were taken at intervals of 6 months or longer. Definite progressive changes took place leading to a characteristic shape, size and position of the heart and aorta on the roentgen-ray picture and also leading to definite changes in the size and shape and sign of the Q R S-T waves in the electrocardiogram. These final changes are specific for long standing cases of hypertension.

The characteristic x-ray film in hypertension shows a sthenic or hypersthenic chest; a hypertrophied left ventricle, usually a concen-

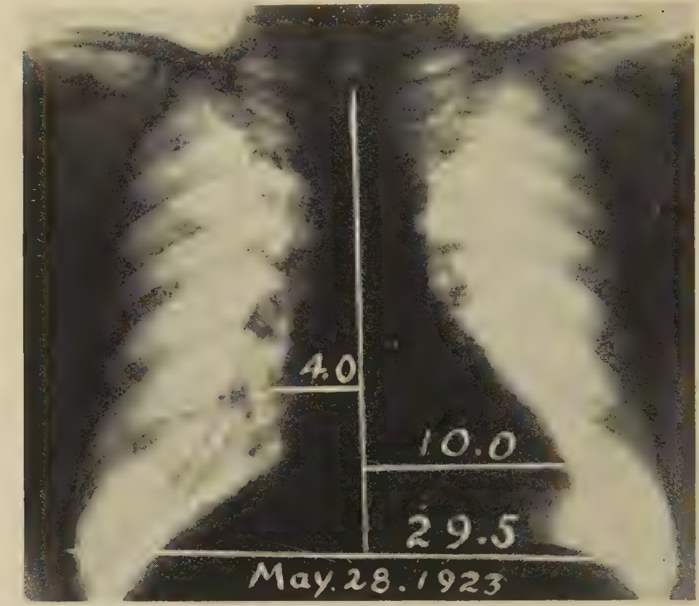


FIG. 1.

a. Bl. pr., 158/100 May 28, 1923. A practically normal heart. Slight hypertrophy left ventricle.

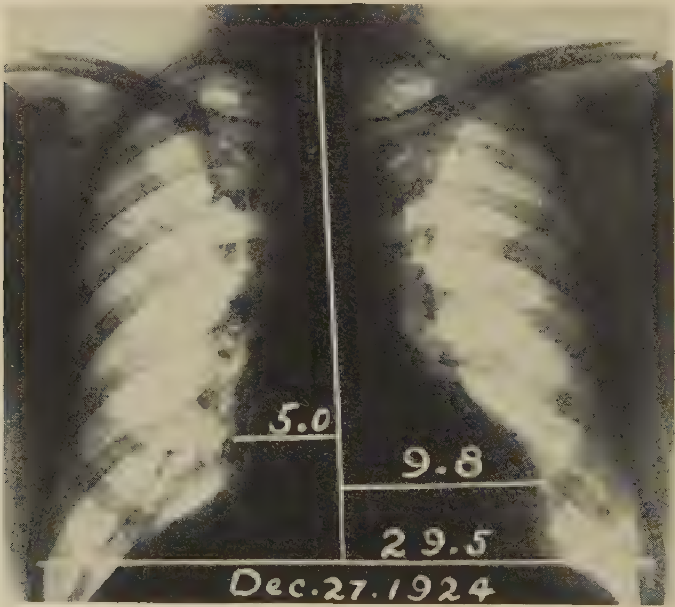


FIG. 1.

b. Bl. pr. 166/100 Dec. 27, 1924. Heart enlarging, hypertrophy of left ventricle. Tortuous aorta, prominent aortic knob.

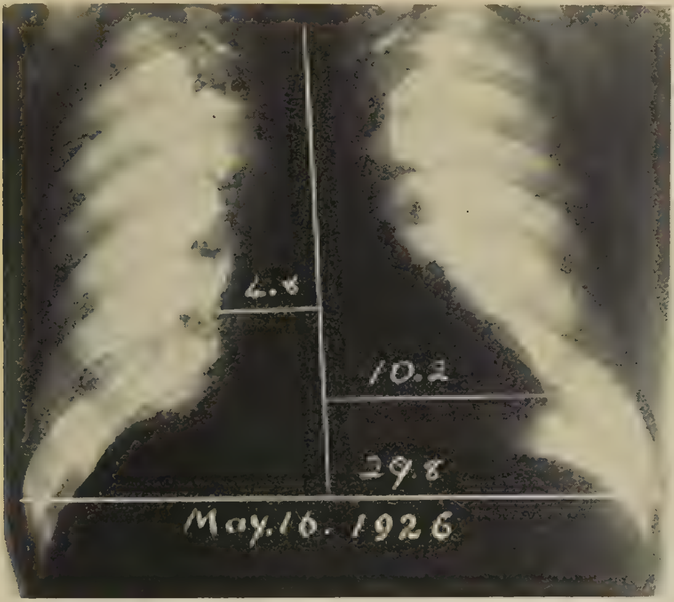


Fig. 1.

c. Bl. pr. 160/100 May 16, 1926. An increased left ventricular hypertrophy.

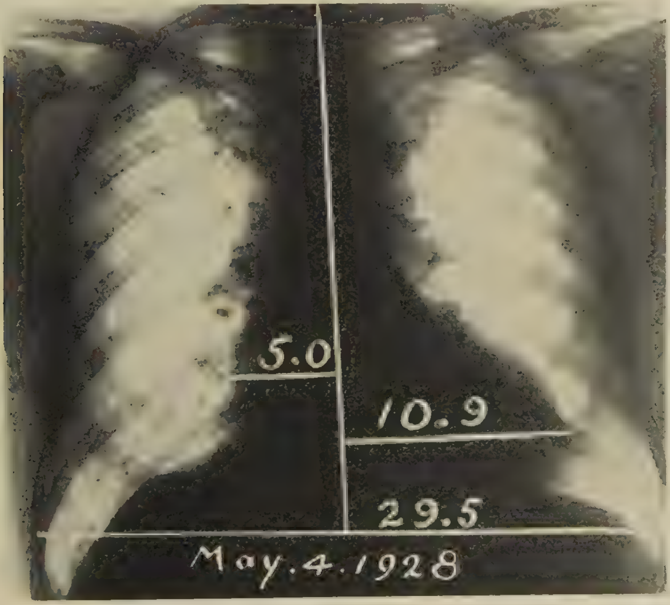
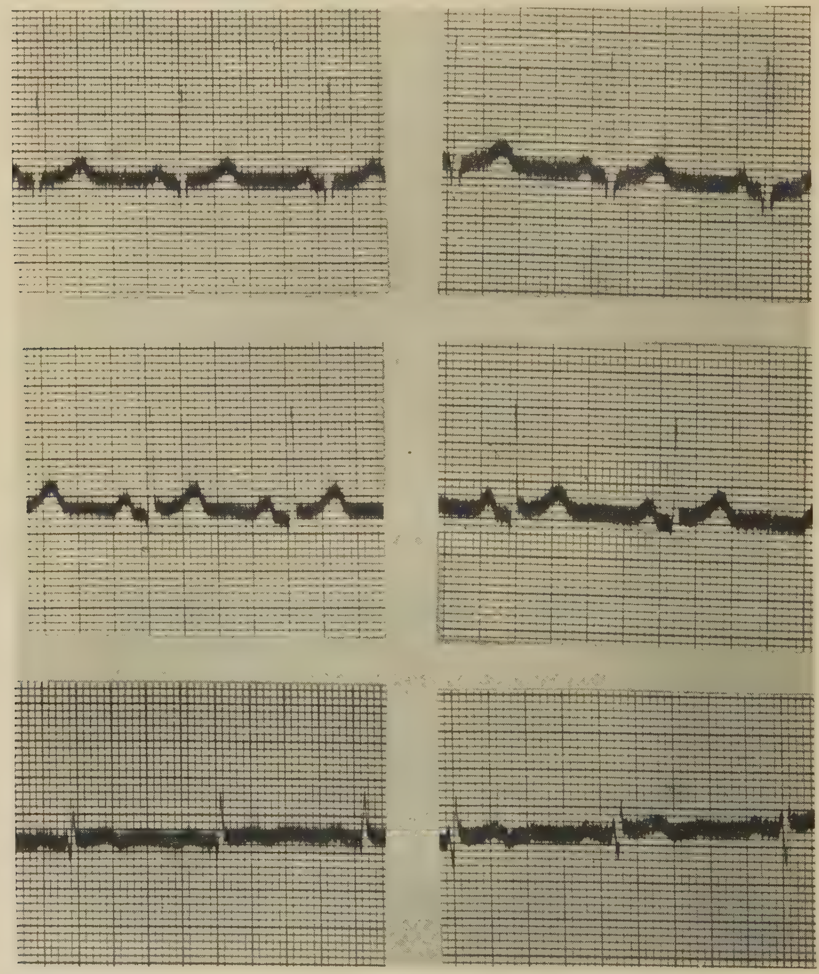


Fig. 1.

d. Bl. pr. 170/110 May 4, 1928. A marked left ventricular hypertrophy with rounded apex.

trically hypertrophied left ventricle; an enlarged heart; a dilated and tortuous aorta. The development of this heart and aorta is gradual. At first the heart and aorta are of practically normal size, shape and position. (See Fig. 1a.) A left ventricular hypertrophy then develops and the heart enlarges. (See Fig. 1b.) This development proceeds until an enlarged heart, hypertrophied left ventricle with rounded apex, and dilated, tortuous aorta appear. These changes occurred over a period of 6 years in this particular case. (See Figs. 1a, b, c, d.)



a

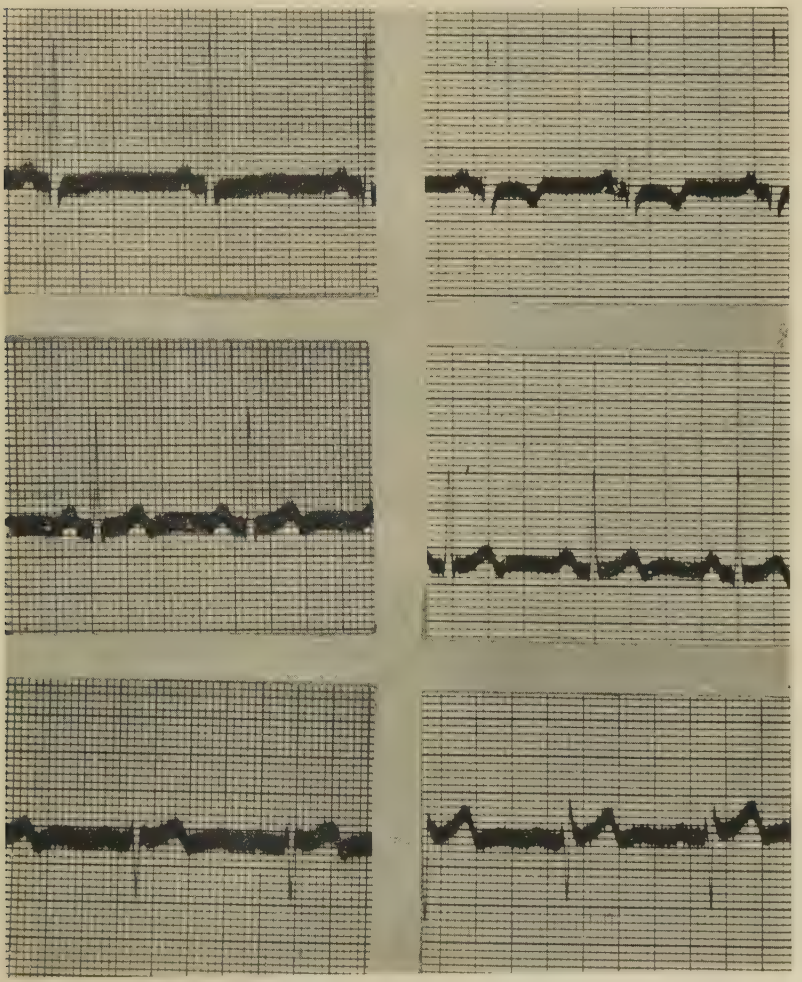
b

FIG. 2.

a. Dec. 23, 1921. Age of patient 59. Normal electrocardiogram.

b. Dec. 29, 1923. Bl. pr. 152/90. A definite left axis deviation and increase in voltage of Q R S group.

Over a period of years a characteristic hypertension electrocardiogram commonly results. This specific electrocardiogram shows a left axis deviation of the Q R S group (left ventricular preponderance), an inversion of the T wave in lead I, high voltage of the R and S waves in which the amplitude is 20 mm, or more in at least one lead, and a diminished or absent S-T transition period. Figure 2a is a normal electrocardiogram taken from the records of



c d

FIG. 2.

c. Nov. 10, 1927. Bl. pr. 170/100. T wave in lead I isoelectric (flat) and further increase in voltage Q R S group.

d. Nov. 1, 1928. Bl. pr. 174/104. T wave in lead I definitely inverted, with still further increase in voltage of Q R S group.

a woman of 59 years of age, Dec. 23, 1921. Figure 2b, Dec. 29, 1923, shows the development of a left axis deviation and an increase in amplitude of the R and S waves. Figure 2c, Nov. 10, 1927, illustrates a further increase in voltage of the Q R S group and a flattening of the T wave in lead I; figure 2d, Nov. 1, 1928, illustrates a further increase in voltage of the R and S waves with the T wave in lead I definitely inverted. These records cover a period of 7 years.

4308

The Transplantation of Mammalian Tissues into Amphibian Tadpoles.

MALCOLM E. LITTLE. (Introduced by Robert Chambers.)

From the Department of Biology, Washington Square College, New York University.

Experiments were undertaken to test the effects of heteroplastic grafts in animals as widely separated as mammals and amphibia. Testicular and thyroid tissues from albino rats were placed in the larvae of *Rana catesbiana,* and other experiments are in progress with both embryonic and adult tissues.

In the first series of grafts no effort was made to control temperature, the experimental animals being left at room temperature of approximately 20°, subject to diurnal fluctuations. The tissues to be grafted were removed aseptically, and simply placed within the coelomic cavity of the tadpole, the coils of the intestine keeping the transplant in place against the peritoneum of the body wall. The operative animals were kept in tap water.

Histological preparations of the tissues show that after 2 days the testicular grafts become adherent to the body wall, and embryonic connective tissue cells from the tadpole begin to invade the transplant. Growth is more rapid near a broken surface, *i. e.,* at the point where the graft lies near the incision in the body wall. At 4 days, encapsulation by the host is well under way, with an interlacing of connective tissue fibers between the tunica of the testis and the peritoneum of the host. The germinal epithelium shows some degeneration but mitotic cells are still evident. At this time capillaries from the tadpole enter the transplanted tissue, the vessels following the trabeculae of the invading connective tissue. The blood supply in most cases comes from the body wall, though it may come from the serous coat of the intestine.

At 12 days the entire germinal epithelium of the graft has practically degenerated, most of the cells showing a complete lack of stainable chromatin. The ripe spermatozoa are intact, and as no developing spermatocytes are present, it is presumed that the former are the original sperm. In the interstices between the tubules are agglomerations of leucocytes and cells which appear to be testicular interstitial cells. The latter are more numerous than in the normal testis. Their history has not yet been accurately traced.

When the operative animals are kept at 10° C., the entire process is slowed down. The tadpole responds more slowly, the process of encapsulation and vascularization being hardly more advanced at 12 days than in room temperature experiments of 4 days. The degenerative processes of the epithelium within the graft are equally slow, the chromatin material of the peripheral cells appearing normal.

Thyroid grafts present greater difficulties of technique, the minuteness of the transplant preventing it from being held firmly to a vascular surface of the host; and even where this has been accomplished the invasion of capillaries is absent, the tadpole responding by merely encapsulating the foreign tissue. Due to the physiological effects of the thyroid secretion, not more than one-third of a rat's thyroid can be placed in a tadpole. Transplantation of the entire gland resulted in death (in 6 cases) in 40 to 48 hours. Tadpoles into which half a thyroid was grafted lived 9 to 11 days and, at the time of death, had developed pectoral limbs, and the tail was being rapidly absorbed. One-third of a thyroid caused a still slower metamorphosis. The pectoral limbs developed and the animals came frequently to the surface for air, showing that the respiratory system was becoming modified toward the adult type. The tail showed only slight absorption.

In these thyroid experiments the morphological changes were coincident with the changes taking place within the transplanted tissue. For 3 weeks the metamorphic changes proceeded steadily, the legs developing as the head and body assumed the proportions and shape of the adult. During the week following the process of development was much slower and, after the fourth week, no further differential development could be observed. At the end of 6 weeks the transplanted tissues were sectioned and showed that the normal histological structure of the thyroid had entirely disappeared.

This is a preliminary report of extended experiments which are now in progress. Even though it may eventually be shown that the grafted tissue does not continue active growth, the method seems to be of value in testing the effects of mammalian tissues upon tadpole growth and differentiation. The tadpole is little susceptible to either infections or operative shock, and new grafts can be added at will. Judged by the thyroid grafts it would seem that the transplanted tissues deliver the secretions into the blood stream in a more normal manner than when the tissues are fed to the animal, or when their extracts are injected. The effect of some tissues cannot be tested by the feeding method owing to their destructive action of the digestive juices; and an operation once in 3 weeks is preferable to a hypodermic injection each day. Another problem involved is the effect upon the transplanted tissue, and the ease with which the histological changes within the graft can be correlated with the effects upon the tadpole.

4309

Treatment of Dehydration of Diarrhea with Parenteral Fluids. I. Effect on Acid-base Status of Blood.

LYNNE A. HOAG AND ELEANOR MARPLES.
(Introduced by Oscar M. Schloss.)

From the Department of Pediatrics, Cornell University Medical College and the New York Nursery and Child's Hospital.

Relief of the clinical symptoms of dehydration in infants with diarrhea is usually secured by the prompt and energetic administration of parenteral fluids. The assumption is generally made that the disturbed chemical relations in the blood are simultaneously improved. Hartmann,[1] however, has recently presented data from which he concludes that the injection of NaCl solution in severely dehydrated infants may lead to a rising chlorine concentration with a consequent fall in bicarbonate content. He assumes that continuing oliguria must be present to permit the development of this unfavorable result. The explanation offered for the acid-producing power of the neutral salt NaCl is the inability of the body to rid itself of the chlorine-ions by excretion through the kidneys as rapidly as the sodium-ions are lost through the intestine. This theory takes proper cognizance of the seriousness of oliguria[2] but at the same time leads to confusion by assuming that a restoration of urine flow is impossible—an assumption which we believe is untenable.

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[1] Hartmann, A. F., *Am. J. Dis. Child.*, 1928, xxxv, 557.

[2] Schloss, O. M., *Am. J. Dis. Child.*, 1918, xv, 165.

We studied the acid-base status of the bloods of 14 dehydrated infants who were treated with parenteral fluids sufficient in quantity to produce and maintain diuresis. The routine treatment comprised the use of hypodermoclyses of physiological solutions of NaCl (about 20 cc. per pound of body weight) followed by intravenous injections of 10% glucose solution (about 10-12 cc. per pound of body weight) to assist in diluting the plasma and to promote urine flow. This procedure was repeated as indicated by clinical and laboratory findings. Every degree of dehydration was represented but the majority of the cases were of moderate severity. Four of the 14 infants died.

Blood samples were collected before and at variable intervals during treatment. The specimens were drawn without stasis, were immediately placed under oil and the sera analyzed for total fixed base and the most important acid radicles, including chloride, bicarbonate, protein and phosphate. The acid and base values so obtained were compared by converting them to cc. of 0.1 N acid and alkali solutions.

The adequacy of the parenteral fluid treatment in restoring the excretory function of the kidneys was estimated by measuring the volumes of urine passed by 6 of the infants during 8 collection periods. These volumes varied from a minimum of 200 cc. to a maximum of 520 cc. per 24 hours, the average output being 315 cc. daily. This amount somewhat exceeds that voided by well infants of similar weights while on corresponding diets. It is reasonable to assume that a comparable degree of diuresis was produced in the other patients.

With the institution of diuresis by the treatment outlined above none of the 14 infants showed an initial lowering of his serum bicarbonate content. The concentration of chlorine in the serum decreased in 10 of these 14 patients. It is evident then that the great majority of the infants exhibited, even during periods of greatly increased NaCl intake, the 2 chief changes indicative of a return of the blood toward a normal acid-base status. This favorable response was initiated to a surprising degree even in the 2 moribund patients who survived only 5 and 6 hours, respectively, following admission to the hospital.

Four of the 14 infants showed moderate increases in chlorine concentration in the first blood samples taken after treatment. In succeeding analyses, 3 of these 4 infants showed a fall in the concentration of chlorine. No further chemical data were secured from the fourth infant because of his rapid clinical recovery. It is important to emphasize again that in every one of these 4 infants the bicarbonate content rose slightly in spite of the increased chlorine-ion concentration. This rise in bicarbonate was rendered possible by an increase in the total base content and a fall in the protein concentration.

Our study reveals that when diuresis was successfully established and maintained there is no evidence to support the view that injections of sodium chloride solutions in dehydrated infants further deplete the lowered alkaline reserve.

4310

Treatment of Dehydration of Diarrhea with Parenteral Fluids. II. Effect on Excretion of Fixed Acid and Base.

LYNNE A. HOAG AND ELEANOR MARPLES.
(Introduced by Oscar M. Schloss.)

From the Department of Pediatrics, Cornell University Medical College and the New York Nursery and Child's Hospital.

Hartmann[1] believes that diarrhea increases the relative loss of fixed base* from the body, first because there is a less complete resorption of alkaline intestinal secretions, and second, because with the accompanying oliguria there is a decreased excretion of fixed acids.* He states that the parenteral injection of NaCl solution may augment the accumulation of fixed acids in the blood by the retention of chlorine-ions leading to a reduction of the plasma bicarbonate content. He cites that portion of the data published by Holt, Courtney and Fales[2] which shows that loose stools contained much larger quantities of fixed base, especially sodium and potassium, than did normal stools. He failed, however, to point out the fact that the excretion of the fixed acids increased in a proportionate manner. Furthermore, the data are unsuited to an adequate consideration of the total loss of acid and base from the body because of the lack of published analyses of the urines.

In an attempt to add to the available data we determined the quantities of fixed acids and base in both the urine and stools of 6 infants with diarrhea in 8 periods of active treatment with parenteral fluids. These fluids consisted of hypodermoclyses of isotonic NaCl solution followed usually by the intravenous injection of 10% glucose solution. Collections of the excreta were begun at varying intervals following the first treatment and were continued for as long as 48 hours whenever circumstances permitted.

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[1] Hartmann, A. F., *Am. J. Dis. Child.*, 1928, xxxv, 557.

* The term ''fixed base'' refers to sodium, potassium, magnesium and calcium. The fixed acids are chloride, phosphate and sulfate. In this report comparison is made between the 2 calculated as cc. of 0.1 N acid and alkali solutions.

[2] Holt, L. E., Courtney, A. M., and Fales, H. L., *Am. J. Dis. Child.*, 1915, ix, 213.

As a basis of comparison, a similar study was conducted on 3 normal untreated infants in 4 collection periods. A normal preponderance of fixed base over fixed acid was apparent in the total excreta of all 3 infants. This relation is most simply expressed as an acid:base ratio. The average of the 4 periods is 1 part of fixed acid to 1.48 parts of fixed base. This figure is derived from the following individual values: 1.45, 1.44 and 1.46 for the periods during which whole lactic acid milk was fed, and 1.55 during the period of protein milk intake. These ratios are surprisingly similar to that existing in normal plasma—105 cc. of 0.1 N fixed acid to 150 cc. of 0.1 N fixed base per 100 cc., a ratio of 1:1.43.

The analyses of the excreta from the patients with diarrhea showed great variations in the acid:base ratio. The average of the 8 periods was 1:1.35. The outstanding fact is that these patients with dehydration, undergoing treatment with parenteral fluids consisting largely of NaCl solutions, excreted relatively more fixed acid than the normal infants. The most striking example of this high acid excretion was furnished by an infant showing rapid clinical improvement. His acid:base ratio was 1:1.01. The maximum preponderance of base (ratio 1:1.54) was seen in a patient rapidly approaching death. Even in this instance the ratio did not exceed that found in a normal infant receiving the same diet.

These results do not correspond to Hartmann's prediction concerning the effect of NaCl solutions on the fixed acid and base excretion of dehydrated infants. We believe this discrepancy to be due to the fact that his explanation presupposed the existence of continuing oliguria and our results were secured during periods of well-sustained diuresis. The successful restoration of urine flow is well indicated by the fact that the daily urine volumes varied from 200 cc. to 520 cc. in these 6 infants whose weights varied from 6 to 11 pounds.

It may be concluded from these data obtained from 6 infants with diarrhea that active treatment with isotonic NaCl solution in combination with hypertonic glucose solution, administered to the point of complete restoration of urine flow, did not disproportionately increase the excretion of fixed base.

4311

Crystalline Principles From Ch'an Su, the Dried Venom of the Chinese Toad.*

K. K. CHEN AND H. JENSEN.

From the Laboratory of Pharmacology, Johns Hopkins University.

In the river and lake regions of China there lives a large toad which secretes a whitish, slimy venom in some superficial organs located behind the eyes. These structures may be similar to the "parotid glands" and other smaller glands of the skin of the tropical toad, *Bufo agua*, the secretions of which were studied by Abel and Macht.[1] When the venom is collected by irritating the animal and is allowed to dry spontaneously, it is known as Ch'an Su.[2] This has been used empirically in Chinese medicine for hundreds of years. It has been employed in the treatment of canker sores, sinusitis, and many local inflammatory conditions, in the relief of toothache, and in the arrest of hemorrhages from the gums.[3] When administered internally in the form of a compound pill, it is said to be able to break colds.

Ch'an Su has been a subject of study by Japanese investigators[4, 5, 6, 7] under the name of "Senso" during the last few years. Their chemical results, however, do not agree, and their published pharmacological data can therefore only be accepted with reservation.

During 1923-25, one of us (Chen) made a preliminary study of several Chinese drugs in China. Ch'an Su was one of them. The potent action of its alcoholic and aqueous extracts on the cardiovascular system was then observed. Owing to the extensive work on ephedrine, a thorough investigation was postponed until in 1928. With the assistance of the Council on Pharmacy and Chem-

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* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

[1] Abel, J. J., and Macht, D. I., *J. Pharm. Exp. Ther.*, 1911-12, iii, 319.

[2] Ch'an means the toad, and Su the venom. They are spelt according to the Mandarin pronunciation. See Y. W. Lee's "A New Chinese-English Dictionary," Shanghai, the Commercial Press, Ltd., 1918, 566 and 657.

[3] Li, S. C., *Pentsao Kang Mu*, 1596 A. D., Chap. 42.

[4] Hayashi, H., *Deut. Med. Wchnschr.*, 1911, xxxvii, 624.

[5] Shimizu, S., *J. Pharm. Exp. Ther.*, 1916, viii, 347.

[6] Kodama, K., *Acta Scholae Med. Univ. Imp. Kioto*, 1920, iii, 299; 1921-22, iv, 201 and 355.

[7] Kotake, M., *Liebigs Ann. Chem.*, 1928, cdlxv, 1.

istry, American Medical Association, a supply of 1729 gm. of Ch'an Su was purchased from Tung Jen T'ang, Peiping, one of the oldest drugstores in China.[8] In the present study, Jensen was responsible for the chemical side and Chen for the pharmacological and pharmacognostical side. Four crystalline bodies have been separated so far. They can be characterized as follows.

1. *Cholesterol,* M. P. 146° C. (uncorrected), gives all the known color reactions for cholesterol. Spectroscopic examination showed an ergosterol content of 2 parts per thousand. The product, after irradiation, is now being subjected to biological tests for antirachitic elements.

2. *Bufagin,* M. P. 217° C. (uncorrected), is easily soluble in alcohol and chloroform, soluble in ether, and gives typical color reactions with chloroform plus concentrated sulphuric acid, and with acetic anhydride plus concentrated sulphuric acid. Abel was the first to name and isolate bufagin in crystalline form from *B. agua.*[1] The bufagin from Ch'an Su has a toxic action on the heart. When the frog's heart was perfused by the method of Howell and Cooke,[9] recently elaborated and extensively used by Sollmann and Barlow,[10] a concentration of 1:100,000 caused a brief acceleration followed by a rapid decrease in rate and amplitude. Relaxation became more and more incomplete until finally the ventricle stopped in systole. More dilute solutions depressed the contractions and rate. Auricular fibrillation and incomplete block were observed with a concentration of 1:10,000, and occasional extrasystoles with that of 1:20,000. A dilution of 1:10,000,000 had no effect. The M. S. D. (Minimal Systolic Dose) in frogs as determined by the one hour method was found to be 0.000045 gm. per gm. of body weight. In pithed cats, 1-1.5 mg. of bufagin proved fatal, due to the cardiac collapse. Sublethal doses had a prolonged pressor action.

3. *A Nitrogen-Containing Compound,* M. P. 200° C. (uncorrected), is soluble in chloroform and alcohol but insoluble in ether, produces color reactions with acetic anhydride plus concentrated sulphuric acid, and gives a positive Sakaguchi's test[11] probably due to the presence of arginine in the molecule. This is an entirely new principle isolated from Ch'an Su and possibly corresponds to the "bufotoxin" which Wieland[12] separated from the European toad,

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[8] Chen, K. K., *Ann. Med. Hist.*, 1925, vii, 103.

[9] Howell, W. H., and Cooke, E., *J. Phys.*, 1893, xiv, 198.

[10] Sollmann, T., and Barlow, O. S., *J. Pharm. Exp. Ther.*, 1926, xxix, 233.

[11] Sakaguchi, S., *J. Biochem. Japan*, 1925, v, 25.

[12] Wieland, H., and Alles, R., *Ber. deut. chem. Gesell.*, 1922, lv, 1789.

Bufo vulgaris. The substance is more toxic than bufagin. Solutions of 1:1,000,000 and higher concentrations produced in the frog's heart a primary acceleration soon followed by a decrease in rate and amplitude with the occurrence of ventricular extrasystoles, auriculo-ventricular block as shown by the 2:1 rhythm, arrhythmia, ventricular intermittence and systolic standstill, while the auricles continued their bradycardia until final arrest was brought about. Concentrations of 1:2,000,000 to 1:10,000,000 caused some depression of the contractions and the rate. The M. S. D. in frogs was determined to be 0.000005 gm. per gm. of body weight. In pithed cats of ordinary size, 0.5-1.0 mg. caused death. Smaller quantities also raised the blood pressure, but not so much as bufagin.

4. *Epinephrine*, M. P. 212° C. (uncorrected), gives the typical color reactions with ferric chloride. This is the first time that this principle also has been obtained in crystalline form from Ch'an Su. Its presence makes the traditional belief justifiable that Ch'an Su stops hemorrhages, and shrinks the nasal mucosa to drain the infected sinuses. Abel[1] discovered epinephrine in the secretions of *Bufo agua* some 18 years ago. The epinephrine prepared from Ch'an Su reproduced all the known physiological responses in the animal body. When assayed biologically in pithed cats, it was proved to have exactly the same pressor activity as a commercial specimen.

Detailed accounts of this work will appear elsewhere.

4312

Influence of Cathode Rays on Leucocytes of Rabbits.

WILHELM STENSTROM, CHARLES H. WATKINS, CARL E. NURNBERGER AND MADELEINE FALLON. (Introduced by F. H. Scott.)

From the Laboratory of Biophysics and the Medical Service of the University Hospital, Minneapolis, Minn.

It is well known that leucocytes are destroyed to a considerable extent if a large portion of an animal is exposed to about an erythema dose of roentgen rays which penetrate into the tissues under the skin. Bucky[1] has stated that very soft roentgen rays, "grenz rays," have a different effect on the leucocytes. According to him, a considerable portion of the leucocytes disappear from the circulating blood immediately after a small area of the skin has been exposed to these rays, which are all absorbed in the first millimeters of tissues, and reappear after a few hours. Cathode rays produced with less than 200 kilo volts are absorbed by the first millimeter of tissue. A very intense dose given to the skin will, therefore, not directly injure the underlying tissues. Such an intense dose can be given in a few seconds. Due to the rapid destruction of tissues a considerable amount of organic substances may be liberated. It is possible that they will be taken up partially by the skin capillaries and thus become mixed with the blood. What effect they may have on the system and on the blood itself is difficult to predict. It seemed likely that the effect should be similar to, though perhaps more intense than the effect of "grenz rays." We decided to study the effect on the leucocytes of rabbits and this is a preliminary report of the first results obtained.

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[1] Bucky, G., *Am. J. Roentg.*, 1927, xvii, 645.

We are obliged to Dr. W. Coolidge of the General Electric Company for supplying us with a tube of the construction which he has described in earlier papers,[2] and to the University of Minnesota for appropriating a special fund for needed equipment. The tube is connected to a system of vacuum pumps and has to be evacuated each time it is being used.

During the exposure of the first group of 3, we used 180 k.v., 0.5 m.a. and 1 cm. distance from the window of the cathode ray tube to the irradiated area which was on the inside of the right ear. The rabbit was placed behind a lead screen 1/16 in. thick. A square hole was cut in the lead and the ear was flattened out over this opening and fastened to the lead by strips of adhesive tape. Rabbit No. 1 had an area of 2x2 cm. exposed for 4 sec., rabbit No. 2 an area of 2x2 cm. exposed for 8 sec., and rabbit No. 3 an area of 3x3 cm. exposed for 8 sec.

For the second group of rabbits one (No. 3) was used for control. It was treated in exactly the same way as the other 2 rabbits with the exception that a piece of lead was placed in front of the hole in the lead screen during the "treatment". Instead of 180 k.v. we used 135 k.v. this time while the current was the same as before, 0.5 m.a. The distance from the window to the irradiated area was reduced to ½ cm. and the exposed area was kept at 3x3 cm. Rabbit No. 1 was irradiated for 10.9 sec. and rabbit No. 2 for 19 sec.

After normal counts were established for each rabbit, the total leucocyte count was taken and a differential count was made as follows: immediately before exposure to the cathode rays, 10 minutes after exposure and at hourly intervals during that day until the leucocyte count became constant, and once daily thereafter until the leucocyte count returned to normal.

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[2] Coolidge, W. D., *J. Franklin Inst.*, 1926, 693.

The effect of irradiation may be divided into 2 portions, the immediate reaction and the sustained reaction. In the first series the immediate reaction is characterized by a marked increase above normal, returning to normal in a few hours. The differential showed a rapid relative decrease and an absolute increase in the lymphocytes, while the neutrophiles showed both an absolute and relative increase. At the end of the immediate reaction the absolute and relative values of both the lymphocytes and the neutrophiles were practically normal. Three hours after irradiation the basophiles showed a slight relative and marked absolute increase, which at the end of the immediate reaction was still far above normal. The monocytes showed a relative and an absolute decrease throughout the immediate reaction.

The sustained reaction in the first series is characterized by a steady increase in the total number of leucocytes reaching a peak on the fifth day following irradiation, and gradually returned to normal about 10 days later. The lymphocytes and neutrophiles remained relatively normal throughout but showed an absolute increase which decreased in proportion to the decrease in the total count. The basophiles, however, showed both a relative and an absolute increase for a period of 8 days following irradiation and gradually decreased to normal. The monocytes showed both a relative and absolute decrease throughout the period of observation.

In the second series the immediate reaction also showed a marked increase above normal which returned to normal in about 5 hours. The lymphocytes showed at first a marked relative decrease and an absolute increase, but after the end of 5 hours there was a relative and absolute decrease throughout the rest of the immediate reaction. The neutrophiles on the other hand showed a marked relative and absolute increase during the entire immediate reaction. The basophiles also showed a marked relative and absolute increase, which returned practically to normal at the end of the immediate reaction. The monocytes showed a slight relative increase followed by a decrease, while the absolute value was increased during the first 5 hours and decreased during the remainder of the reaction.

In the sustained reaction 2 days after irradiation there was a slight secondary rise in the total leucocyte count with a slight relative decrease and absolute increase of the lymphocytes and a relative and absolute increase of the neutrophiles with a relative and absolute decrease of both basophiles and monocytes. From the third day after irradiation to the end of the period of observation the total leucocyte and differential counts were normal with the exception of a slight relative and absolute decrease in the monocytes.

The rabbit which was used as a control showed the differential normal throughout the immediate reaction period. On the next day, however, the total leucocyte count was increased with an increase in neutrophiles, but the characteristic basophilia did not occur. From this time the total leucocyte and differential counts were normal. This temporary rise in the leucocyte count was probably due to an infection, following thrombosis of the lateral vein of the left ear.

4313

Duration of Anticoagulant Action of Heparin in Vivo in Relation to Dosage.

PAUL GROSS.* (Introduced by E. E. Ecker.)

From the Department of Pathology, School of Medicine, Western Reserve University, Cleveland, Ohio.

Reed[1] determined experimentally the "minimum dependable dose" of heparin as an anticoagulant. Takuji Shinoya,[2] upon injecting heparin intravenously into dogs, commented on the rapidity with which the coagulation time increased and then returned to normal. In the following experiments determinations were made of the rate of appearance and disappearance of the anticoagulant action of heparin *in vivo*.

Heparin was injected in varying amounts into the ear veins of rabbits, and the blood was collected from cut veins of the opposite ears. One method of determining clotting time which was used is as follows: Drops of blood were collected at intervals of 20 seconds or more upon glass slides cleaned with alcohol and ether. The time when each drop fell upon the slide was noted. Only blood which exuded and dropped readily was used. The blood was termed clotted when, upon being moved with a wire loop, clear or nearly clear serum was left behind. The slides were kept in a moist chamber at room temperature. A simpler method, used in the later series of experiments, is the capillary tube method. These 2 methods were used interchangeably without variations in results. The data of such an experiment are given in Table I.

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* Crile Research Fellow in Pathology.

[1] Reed, C. I., *Am. J. Physiol.*, 1925, lxxiv, 79.

[2] Shinoya, T., *J. Exp. Med.*, 1927, xlvi, 21.

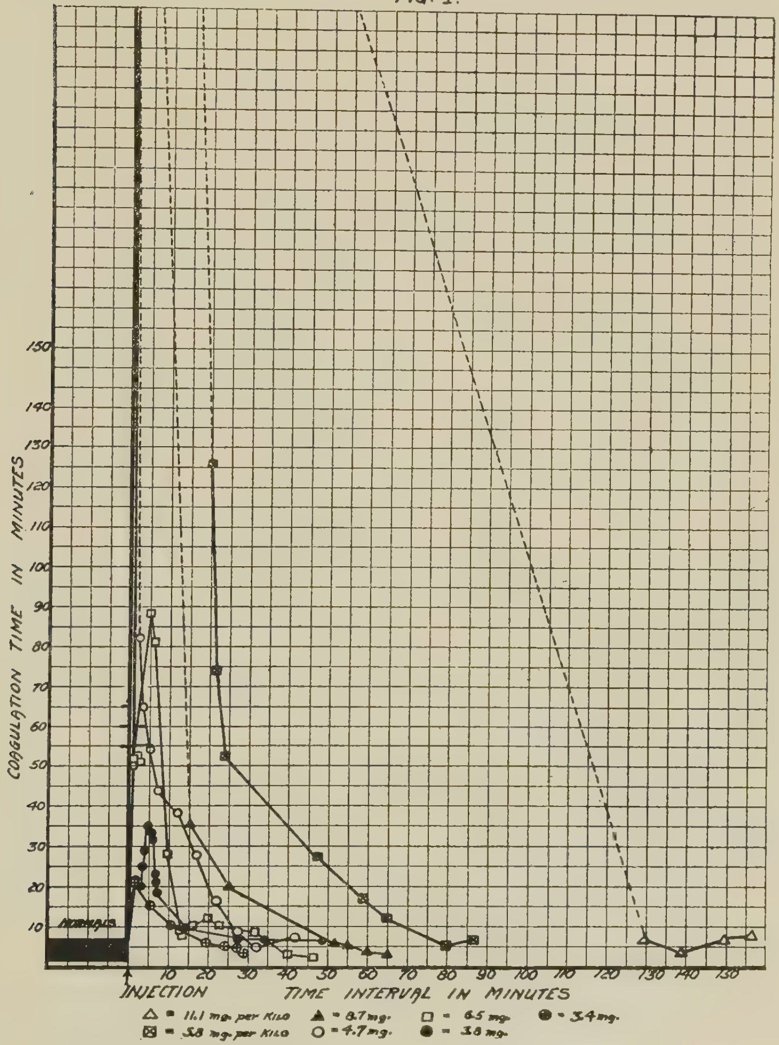
TABLE I.—*Anticoagulant Effect of Heparin In Vivo.*
Intravenous injection of 10 mg. of heparin into a rabbit weighing 1725 gm.

Time	11:54	11:55	12:09	12:10	12:22	12:22	12:38	12:42	12:44	12:46	1:19	1:20	1:27	1:42
Clotting Time in minutes	5½	6	5½	6½	Inject 10mg. hep'n	No clotting in 4 hours		126	74	52	27⅓	17	12	5

The clotting time was increased to more than 4 hours as soon as 30 seconds after the injection of heparin. Within 20 minutes after the injection, the clotting time began to return toward normal. The normal clotting time was reached within one and a half hours from the time of injection.

Upon plotting a number of curves from data similar to that in Table I, but for varying dosages of heparin, a similarity in the configuration of the different curves is observed (see Fig. 1).

FIG. I.

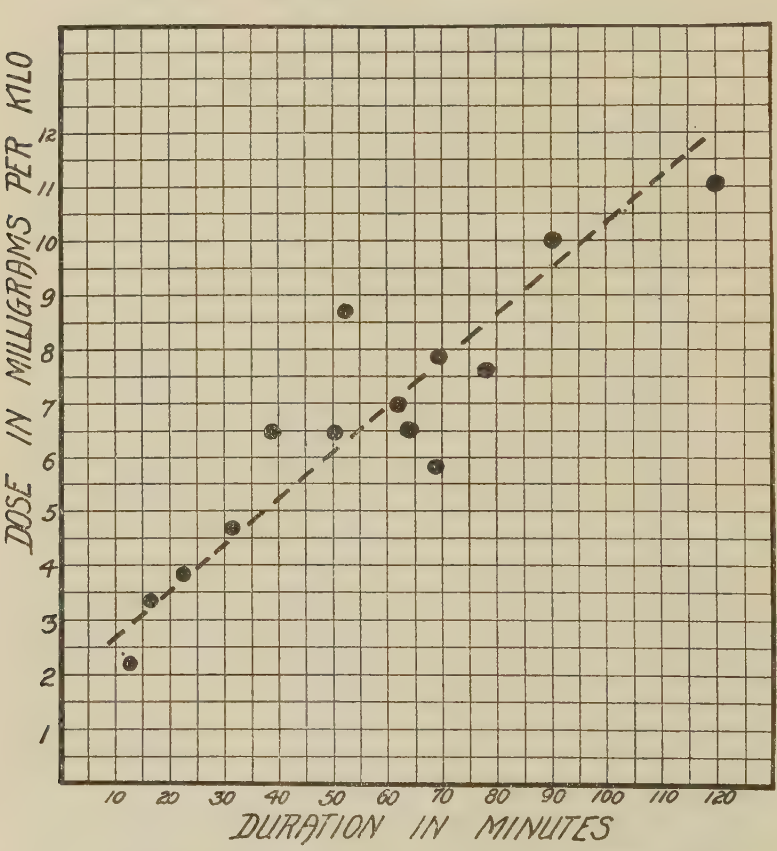


ANTICOAGULANT EFFECT OF HEPARIN IN VIVO

The interrupted lines connect points of definite coagulation time with points where the blood was last found to be incoagulable. The broad line labelled *normals* represents the range of variability in the normal coagulation time of different animals taken for periods as long as 2 hours.

If the duration of the heparin action be plotted against the dosage employed, a representation such as in Fig. 2 is obtained. The moment at which the clotting time fell to within 2 minutes of the normal, was arbitrarily designated as the end of the heparin effect.

FIG. II



RELATION OF DOSE OF HEPARIN TO DURATION OF ITS EFFECT

Summary: 1. The intravenous injection of heparin is followed by an immediate increase in clotting time. Expressed graphically, this rise is practically perpendicular. The rate of return toward the normal clotting time is very rapid for one third of the duration of the heparin action, and subsequently, more slowly. 2. Graphic expressions, for different dosages of heparin, of the changes in clotting time are similar. 3. In general, there is an approximate relationship, as expressed by the graph, between the dose of heparin per kilo of body weight and the duration of the heparin effect in rabbits.

4314

Blood Phosphates in Milk Fever.

PIERRE A. FISH.

From the Laboratory of Veterinary Physiology, Cornell University, Ithaca, N. Y.

The work of Little and Wright,[1] confirmed in our laboratory,[2] and the work of Dryerre and Greig[3] have shown that there is a marked diminution in the amount of blood calcium in cows "down" with milk fever. It occurred to the writer that since calcium and the phosphates are intimately associated in many of the processes of body metabolism, the phosphates of the blood might also be affected.

Work along this line was begun in September, 1927, and, up to this time, 18 cases of milk fever have been tested. The results have been uniform in showing a subnormal amount of the inorganic phosphate and acid soluble phosphorus by the Fiske-Subbarow method prior to the inflation of the udder. In another investigation, we have found the average for the inorganic phosphates in normal milk cows to be 4.74 mg. per 100 cc. of serum, and for dry cows 6.45 mg.-100. The average of the acid soluble phosphorus for the milk cows was 7.95 mg.-100 and for the dry cows 9.23 mg.-100. The average of the inorganic phosphorus in the dry cows was 50% higher than that of the milk cows, and the acid soluble phosphorus was likewise higher, 16%, than in the milk cows. The calcium content was found to be the same in both groups of animals.

The accepted remedy for milk fever is inflation of the udder. The effects are usually rapid, the animal recovering from its collapse or coma, and standing on its feet again, often within a period of 6-8 hours. The first blood sample was always taken just prior to udder inflation and the second sample, and occasionally a third sample, were taken at various intervals after inflation. In this way it was possible to determine approximately the time required for the phosphates to get back to their normal standard. The average of the preinflation sample for the inorganic phosphates for the 18 milk fever cases was 2.28 mg. per 100 cc. The average for the preinflation samples for the acid soluble phosphorus—17 cases—was 6.49 mg. Comparing these averages with the normal for the milk cow, it is found that there is in the inorganic phosphates a decrease of about one-half in the milk fever cases, and but a moderate decrease in the acid soluble phosphorus.

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[1] Little and Wright, *Brit. J. Exp. Path.*, 1925, vi, 129.

[2] Fish, P. A., *J. Am. Vet. Med. Assn.*, 1928, lxxiii.

[3] Dryerre, H., and Greig, R., *Dumfries and Galloway Vet. Med. Assn.*, July 7, 1928.

The effect of udder inflation upon the rise of the blood phosphates is indicated in the following graphs:

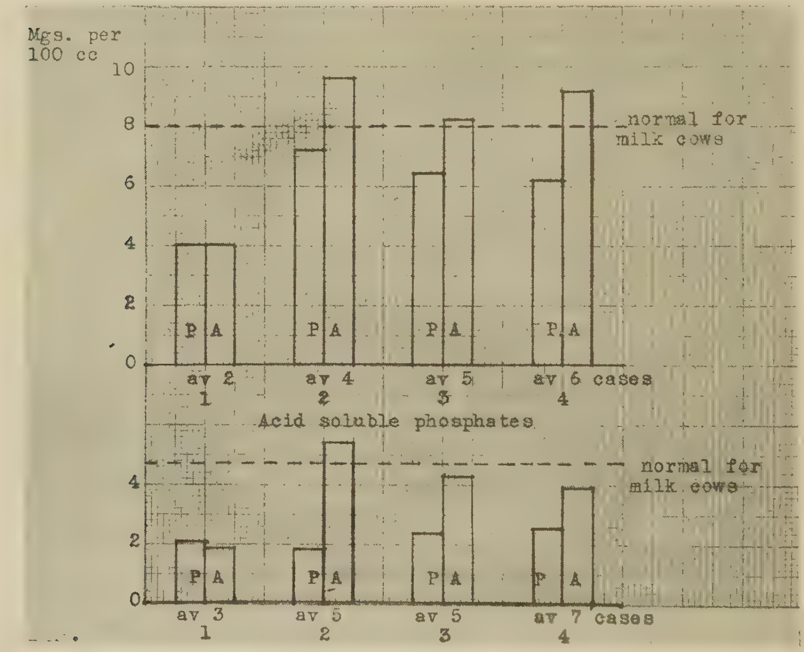

FIG. 1. *Inorganic Phosphates.*

P = preinflation tests. A = tests after udder inflation.

In graph 1, A = 10-20 minutes after inflation. In graph 2, A = 6-8 hours after inflation. In graph 3, A = 10-18 hours after inflation. In graph 4, A = 24-36 hours after inflation.

The time periods refer to both inorganic and acid soluble phosphorus.

The height of the preinflation tests in the graphs does not represent the average of the total number of cases, but the average for the number of animals in each specific group. In the first group, 10-20 minute period, it is very desirable to test a larger number of animals before accepting the slight decrease in the amount of the phosphates as a constant feature.

As a general conclusion from a study of the graphs it would appear that there is a continued slight decrease in the amount of the inorganic phosphates for 10-20 minutes after inflation; but that in the 6-8 hour period they have risen to normal or a little over, with a slight decrease below normal in the 10-18 and 24-36 hour periods. The acid soluble phosphorus shows a somewhat similar rise and fall for the same periods except that in the 10-20 minute period the amount was about the same as in the preinflation tests and the fluctuations in the subsequent periods were all a little above the normal.

Whether the diminished amount of the phosphates is correlated with the decreased amount of calcium in the blood of the milk fever cases or whether the decrease of each is independent of the other, or whether the calcium or the phosphates may be the more important factor in the phenomena associated with the disease, cannot be stated at the present time.

In our experience the blood calcium requires a considerably longer period than the phosphates to reach its normal standard. It is perhaps significant that the recovery of the cow seems to be coincident with the return of the blood phosphates to their normal standard.

4315

Anterior Lobe, the Thyroid Stimulator, V. Basal Metabolism.

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*From the Anatomical Laboratory, University of Maryland Medical School.**

If anterior lobe of the hypophysis is the activator of the thyroid gland, it should raise the basal metabolism. We made 96 Winkler-tests to determine the oxygen consumption of larvae of *Ambystoma tigrinum,* 37 tests of normal control larvae, 33 tests of larvae injected with an extract of bovine anterior lobe powder, which was prepared in our own laboratory, 16 tests of larvae which had been fed this powder, 7 tests of larvae which were fed Armour's anterior lobe powder and 3 tests of larvae fed posterior lobe powder prepared in our own laboratory.

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* We take pleasure in expressing our gratitude to the Trustees of the "Elizabeth Thompson Science Fund" of Harvard University, for the support received from this fund.

Larvae of the Utah axolotl were tested without anesthesia. The average oxygen consumption of the injected animals was found 30% higher than that of the controls.

Larvae of the eastern race of *Ambystoma tigrinum*† were tested without anesthesia. The average oxygen consumption of the injected animals was 51% higher than that of the controls.

Larvae of the eastern race of *A. tigrinum* were tested in chloretone anesthesia. The average oxygen consumption of the injected animals was 114% higher than that of the controls.

Feeding of the anterior or posterior lobe powders did not change the oxygen consumption.

We expressed oxygen consumption in cc. of oxygen per hour and gram animal weight. But metabolism changes parallel with surface and not with weight. Of 2 species having actually the same oxygen consumption, but different size, the larger one may incorrectly appear to have the lower oxygen consumption, if oxygen consumption is calculated per gram weight. In our experiments all animals were of the same species and, in 2 experiments, of the same brood. The differences in oxygen consumption between the controls and the injected experimentals were considerable and present even if controls and experimentals were of the same size and weight as was often the case. One pair tested on the same day and with the same solutions will illustrate this statement:

Control:	Weight	3.07 gm.	0.199 cc.	oxygen.
Experimental:	"	3.06 "	0.294 "	"

Conclusion: Anterior lobe manufactures a substance which increases metabolism; it is not species-specific and is effective only when injected intraperitoneally, but ineffective when administered *per os*. This finding is in accord with our previous findings according to which anterior lobe forces the thyroid into excessive activity and thereby causes precocious metamorphosis, but is completely ineffective in the absence of the animal's own thyroid gland.

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† We owe the spawn from which these animals were raised to the kindness of Dr. S. C. Bishop, of the University of Rochester, who collected it on Long Island, spring 1928.

4316

The Bactericidal Action of Indole.

R. B. KILBORN, H. B. PIERCE AND R. P. TITTSLER.
(Introduced by R. A. Dutcher.)

From the Department of Agricultural and Biological Chemistry and the Division of Bacteriology, Pennsylvania State College.

Gordon and McCleod[1] have called attention to the fact that there is little or no information to be found in the literature dealing with the antiseptic potency of indole. By experimentation these authors

TABLE I.
Effect of Various Concentrations of Indole on Bacterial Growth.

Organism	Conc. of Indole	Total Count	Organism	Conc. of Indole	Total Count
		per cc.			per cc.
Escherichia coli	1-1000	0	*Aerobacter aerogenes*	1-1000	0
	1-1000	0		1-1000	0
	1-2000	0		1-2000	8,000
	1-2000	580,000		1-2000	4,000
	1-4000	63,000,000		1-4000	95,000,000
	1-4000	64,000,000		1-4000	83,000,000
	1-4000	39,000,000		1-4000	80,000,000
	1-4000	45,000,000		1-8000	450,000,000
	1-4000	60,000,000		1-8000	560,000,000
	1-4000	70,000,000		no indole	600,000,000
	1-8000	250,000,000		no indole	900,000,000
	no indole	370,000,000			
	" "	480,000,000			
	" "	500,000,000			
	" "	520,000,000			
Eberthella typhi	1-1000	0	*Salmonella paratyphi*	1-1000	0
	1-2000	500		1-1000	0
	1-2000	194		1-2000	22
	1-4000	40,000,000		1-2000	0
	no indole	207,000,000		1-2000	195
	no indole	230,000,000		1-4000	42,000,000*
				1-4000	0
				1-8000	270,000,000
				1-8000	210,000,000
				no indole	430,000,000
				no indole	810,000,000
Salmonella schotmulleri	1-1000	2	*Salmonella enteritidis*	1-1000	0
	1-2000	39,000		1-1000	0
	1-4000	87,000,000		1-2000	18,300
	1-4000	74,000,000		1-2000	21,000
	1-4000	80,000,000		1-4000	90,000,000
	no indole	480,000,000		1-4000	98,000,000
	no indole	550,000,000		1-4000	130,000,000
				no indole	300,000,000
				no indole	380,000,000

*Estimated.

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[1] Gordon, J., and McCleod, J. W., *J. Path. and Bact.*, 1926, xxix, 13.

found indole to have an antiseptic effect ranging from 2 to 10 times that of carbolic acid, the efficiency depending upon the kind of bacteria exposed to its action. *B. coli* was one of the most resistant organisms but failed to grow in the presence of 0.1% indole. Gordon and McCleod believe that indole production may well be the mechanism by which *B. coli* maintains its dominant position in the fecal flora. During a study of the production of indole by fecal bacteria, information was desired concerning the effect of indole upon the growth of bacteria.

The medium used for the tests was 2% Witte's peptone water which had been adjusted to yield a pH of 7.6 after autoclaving. A solution of indole 1-1000 was prepared and filtered by pressure through a Seitz filter. Various amounts of this solution were added to 50 cc. portions of sterile peptone water. The flasks of medium were then inoculated with the organism to be studied by transferring a loopful of a 24-hour culture in 2% peptone water. All of the test cultures were incubated at 37° C. for 24 hours after which counts were made by the plating method.

The results obtained are shown in Table I. The growth of all of the microorganisms studied was hindered by the presence of indole in the culture medium. *Escherichia coli* was the most resistant of the organisms studied. Nevertheless its growth was prevented in the presence of 0.1% indole. *Salmonella paratyphi* was apparently very susceptible to the action of indole. Each result shown in the table was obtained by the analysis of separate cultures.

4317

Effect of Echinid Egg-Waters on the Surface Potential Difference of the Sperm.

EMILY B. H. MUDD, STUART MUDD AND ANNA K. KELTCH.

From the Laboratories of Eli Lilly & Co. in the Marine Biological Laboratory, Woods Hole, and the Henry Phipps Institute, University of Pennsylvania, Philadelphia.

The egg-waters of the sand-dollar *(Echinarachnius parma)*, starfish *(Asterias forbesii)*, and sea-urchin *(Arbacia punctulata)*, have been tested by electrophoresis for their effects on the sperm of each of these 3 species. Egg-water was routinely prepared by allowing freshly obtained eggs to stand a half-hour or more in contact with 0.5 M. NaCl adjusted with NaOH to an approximate pH of 8.2.

This solution was filtered and mixed in equal volumes with suspensions of fresh sperm in 0.5 M. NaCl of pH 8.2. In 2 experiments the egg-water and sperm suspensions were both made with sea-water instead of NaCl solution. Later experiments showed that the exposure to 0.5 M. NaCl of pH 8.2 did not kill the sperm or the eggs from which the egg-waters were derived.

For agglutination the egg-water used was the same as in the corresponding electrophoresis experiment, but the sperm were suspended in sea-water. The agglutination technique was essentially that of Lillie and Just.[1]

In the earlier experiments precautions to obtain the sperm and eggs free from perivisceral fluid were not taken; in the later experiments such precautions were taken. Differences in result due to such precautions were not noted.

Electrophoresis was conducted in a Northrop-Kunitz microcataphoresis cell[2] of a type described elsewhere.[3] Observations were made on sperm whose heads were in the focal plane.

Each of the sperm species studied was found to be negatively charged. In Table I are given the mean cataphoretic velocities with their probable errors, and the surface potential differences calculated from these velocities making the assumptions of Northrop and Cullen.[4] The values in Table I are all for control sperm suspensions before contact with egg-water.

TABLE I.

Sperm species.	Suspending medium†	Cataphoretic velocity μ/sec. per volt/cm.	Sperm surface p.d., millivolts
Sand-dollar	0.5 M. NaCl, pH 8.2	1.71 ± .03	21.6 ± .38
Star-fish	" " " "	1.77 ± .05	22.3 ± .63
Sea-urchin	" " " "	2.10 ± .03	26.5 ± .38
Sand-dollar	Sea-water	1.29*	16.3
Star-fish	" "	1.44*	18.1
Sea-urchin	" "	1.75*	22.0

† See Loeb, J., *Am. Nat.*, 1915, xlix, 257.
* 4 determinations only.

The effects of the egg-waters on the sperm surface potential differences are given in Table II.

Sand-dollar egg-water caused a specific *increase* in the negative

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[1] Lillie, F. R., and Just, E. E., "Fertilization" in Cowdry, E. V., General Cytology, Chicago, 1924.

[2] Northrop, J. H., and Kunitz, M. J., *J. Gen. Physiol.*, 1924-5, vii, 729.

[3] Mudd, S., Lucké, B., McCutcheon, M., and Strumia, M., *Colloid Symposium Monographs*, New York, 1928, vi, 131.

[4] Northrop, J. H., and Cullen, G. E., *J. Gen. Physiol.*, 1921-2, iv, 638.

TABLE II.

Egg-water species	Sperm species	Sperm P.D. increased. No. expts.	Sperm P.D. reduced. No. expts.	Average change
				%
Sand-dollar	Sand-dollar	7	1	+20
" "	Sea-urchin	2	4	— 2
" "	Star-fish	4	4	— 2
Sea-urchin	Sand-dollar	10	1	+33
" "	Sea-urchin	7	2	+13
" "	Star-fish	8	2	+20
Star-fish	Sand-dollar	4	3	0
" "	Sea-urchin	0	7	—14
" "	Star-fish	4	3	+ 6

A mean increase in the negative charge is indicated in column 5 as +, a reduction in the negative charge as —.

charge of sand-dollar sperm and was without significant effect on the sperm of sea urchin or starfish. In correlation with this the sand-dollar egg-water caused specific isoagglutination of sand-dollar sperm, to an average dilution of the egg-water of about 1:50.

Sea-urchin egg-water *increased* the negative charge of sea urchin sperm and caused reversible sperm agglutination of the iso-type in an average dilution of the egg-water of about 1:1300. However, sea-urchin egg-water caused an even greater increase in the negative charge of star-fish and sand-dollar sperm, but caused agglutination only of the hetero-type, and this only in high concentrations of the egg-water.

Star-fish egg-waters regularly *decreased* the negative p.d. of the highly charged sea-urchin sperm, showed a somewhat questionable mean *increase* in charge of homologous sperm and showed no mean effect on sand-dollar sperm. Agglutinative effects with star-fish egg-waters were slight.

The only conclusions we care to draw from the work at its present stage is that echinoderm egg-waters contain substances which may combine with substances in the sperm surfaces, and that such combinations can be detected by cataphoresis.

4318

Observations on the Lymphatic Drainage of Joint Cavities.

MILLARD SMITH AND JOHN R. CAMPBELL, JR.

From the Department of Medicine, University Hospital, Ann Arbor, Mich.

The physiology of joints depends at least in part upon the mechanism for fluid exchange in these structures. Considerable attention has been given in the past to the factors which determine the formation and characteristics of intra-articular effusions, but the ways in which they can be removed are imperfectly known. From a consideration of the factors involved in the equilibrium between the capillaries beneath the synovial membrane and a joint effusion containing protein, one may conclude that absorption by way of the capillaries will cease when the filtration and osmotic pressure relations within the capillaries and within the joint space are in equilibrium. Furthermore, such equilibrium relations can become established with fairly large amounts of effusion fluid remaining within the joint. It may be pointed out also that absorption by way of the blood capillaries would be slow even under ideal conditions.

It is the purpose of this communication to direct attention to the lymphatic system as the most important factor concerned in the drainage of joints under both normal and pathological conditions. This is not a new or original concept but it has not received the emphasis in the past which it demands. It is known that the synovial membrane is abundantly supplied with lymphatic capillaries, but we have been unable to find any specific statement about the lymphatic trunks which drain these capillaries.

Utilizing the knee joints of cats we have determined by injections of solutions containing methylene blue that the lymphatic capillaries of the synovial membrane unite in the popliteal space, with a lymphatic trunk coming from the foot and running up Hunter's canal and then into the pelvis, where it ends in a deep subinguinal lymph node situated at the brim of the pelvis just lateral to the mid line. So far as we were able to determine, this trunk drains only the joint structures. The popliteal lymph nodes drain only the skin and soft structures of the lower leg. The same relations obtain in the dog. These observations suggest the possibility of similar findings in the human.

Using Berlin Blue solutions as injection material we found that the lymphatics were not filled until intra-articular pressure was so elevated that the capsule of the joint was greatly distended. Since Berlin Blue is a non-diffusible colloidal dye, this observation contradicts previous ideas about lymphatic connection with the joint space by means of stomata within the synovial membrane. That the lymphatic capillaries finally did fill with the dye when the latter was injected under considerable pressure indicates that the synovial membrane and the lymphatic capillary membrane are somewhat permeable to colloidal substances. We have also noted a definite difference in the rapidity of drainage from the joint space between isotonic and hypertonic NaCl solutions, and at the same time demonstrated that they were drained by way of the lymphatics by adding to the solution enough methylene blue to color the solution.

Since the lymphatic trunk draining the joint had its course along Hunter's canal it seemed improbable that muscular action ("the milking of lymphatic trunks") could account for lymph flow from the joint. We performed a series of experiments to demonstrate that joint motion was responsible for lymph filtration through the synovial membrane and flow along the lymphatic trunk. Chart 1 illustrates the changing pressure relations within the joint during

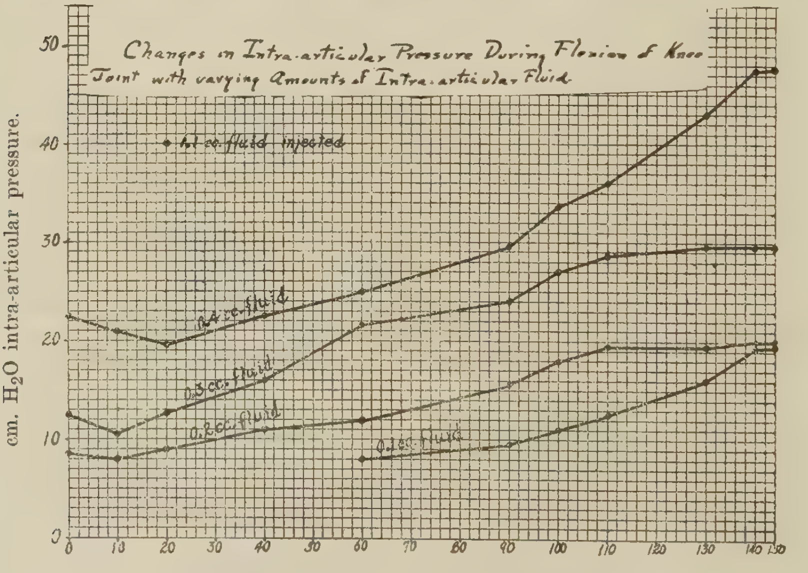

FIG. 1.

Each curve was obtained by means of a water manometer connected to a Luer needle inserted through the patella of the knee joint of a cat. The points on a given curve represent the pressure within the knee joint in terms of centimeters of water pressure at the angle of flexion indicated on the abscissae. In each curve a constant amount of fluid was kept within the joint space and the amount of this fluid is indicated in cubic centimeters on the curve. Flexion was measured by the angle between the limb distal to the knee joint and the line of full extension of the limb.

full range of motion with different amounts of fluid within the joint. The point of least pressure corresponds closely with the position maintained by a limb containing a palpable effusion. By means of a hypertonic saline solution containing methylene blue we demonstrated that drainage was dependent upon joint movement.

These observations indicate that fluids poor in, or, free from colloidal materials will be drained rapidly from the joint space by way of the lymphatics when joint motion occurs. Fluids containing colloidal material which exerts an osmotic pressure may be concentrated until the osmotic pressure of the fluid equals the highest intra-articular pressure attainable by joint motion. Our observations suggest that even colloidal material may filter through the lymphatic capillary membrane when high intra-articular pressures are produced, but it is well known from clinical experience that the absorption of joint effusions is slow when the protein content is appreciably elevated.

4319

Hemorrhagic Glomerular Lesions Produced by Filtrates of Streptococcus Viridans Cultures.

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It is well known that hemorrhagic, glomerulo-nephritis occurs with great frequency in association with *Streptococcus viridans* endocarditis. The glomerular changes are generally regarded as a direct result of damage caused by minute emboli from the vegetations on the heart valves. The following considerations led us to question the assumed embolic nature of the nephritis: (1) If the nephritis associated with *Streptococcus viridans* endocarditis is merely the result of emboli, one might reasonably expect to encounter a similar nephritis quite as commonly in every form of endocarditis characterized by profuse, friable vegetations, regardless of the etiology. This, however, is not the case. (2) There is no relation between the size of the valvular vegetations and the occurrence or degree of nephritis. (3) We have observed florid hemorrhagic nephritis in association with gonococcal endocarditis, in which the vegetations were confined to the right side of the heart, and in which therefore, the capillary bed of the lungs could act as a filter for emboli escaping from the vegetations. It may be remarked that gonococcal endocarditis resembles *Streptococcus viridans* endocarditis in the extraordinary frequency with which it is accompanied by hemorrhagic nephritis. (4) Our microscopic studies failed to convince us that damage to glomerular tufts in the nephritis associated with *Streptococcus viridans* endocarditis is necessarily dependent upon the presence of emboli. (5) The glomerular damage differs in no way from that observed in cases of nephritis in which emboli could have played no rôle.

It seemed possible that the high incidence of nephritis occurring in association with certain forms of bacterial endocarditis might be referable to the action of toxins produced by the microorganisms in question, rather than to emboli. We, have, therefore, carried out experiments with a strain of *Streptococcus viridans* which was obtained from the blood of a patient suffering, in a typical manner, from subacute endocarditis, with evidences of renal involvement.

Briefly, we were able to produce glomerular damage in rabbits by the intravenous injection of bacteria-free filtrates of fresh broth cultures of this organism. The sterility of the filtrates was always proven by culture. The amount of filtrate injected varied between 2 and 20 cc., and the animals were killed on the day following the injection. There was no definite relation between the amount injected and the degree of renal injury produced. Some unexplained individual susceptibility played a greater rôle.

Realizing the sources of error involved in the interpretation of apparent cellular alterations in the glomerular tuft, we have regarded as positive only those cases in which actual hemorrhages occurred into Bowman's capsule and the convoluted tubules. The hemorrhages visible at autopsy in the gross varied in number from only one or two to a profuse peppering of the cortex of each kidney. Microscopically, the hemorrhages were always in Bowman's capsule or the tubules, never in the interstitial tissue, and in all instances the escaped blood was perfectly fresh in appearance. In no case did the control injections of broth alone affect the kidney.

Mention of spontaneous, hemorrhagic glomerulo-nephritis in the rabbit is of excessive rarity in the literature. We have never encountered it in the study of the kidneys of hundreds of rabbits killed for other purposes during the past several years, and our control series of 90 rabbits were free from it. We feel, therefore, that the present experiments demonstrate clearly that, in the Berkefeld filtrates of broth cultures of a pathogenic strain of *Streptococcus viridans* there is something which can produce glomerular dam-

TABLE I.

Material Injected	No. of Animals used	Animals showing glomerular hemorrhages at autopsy	Animals showing no hemorrhages at autopsy	Animals showing glomerular hemorrhages
Living *Strep. viridans*	29	9	20	% 45
Heat killed *Strep. viridans*	23	4	19	21
Berkefeld filtrate of broth culture *Strep. viridans*	79	17	62	28
Broth alone	90	0	90	0

age in the rabbit. As far as we are aware, this is the first time that a condition comparable to acute hemorrhagic glomerulo-nephritis has been produced experimentally by means of a bacterial filtrate.

Whether or not we are dealing with a true toxin cannot yet be stated. We have evidence, however, that the renal damage in our experiments has nothing to do with allergy. We can offer no explanation for the differences in individual susceptibility to the injurious substance in these experiments. The same problem confronts us in cases of infection with the *Streptococcus viridans* in the human being. Our efforts to concentrate the toxic principle of the filtrate and so to obtain, possibly, a higher percentage of instances of renal involvement have not yet been successful. Our experiments planned to subject animals to repeated injections over a period of time are incomplete. In a limited number of experiments in which filtrates of cultures of other bacteria were injected, no renal changes were produced.

4320

Induction of Anesthesia in Man by Intravenous Injection of Sodium Iso-Amyl-Ethyl Barbiturate.

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During the last 6 years the sodium salt of iso-amyl-ethyl barbituric acid has been used extensively as a general anesthetic in animal experiments.[1] The difficulty experienced in preparing strictly uniform solutions of this substance and the tendency of such solutions to deteriorate on standing have heretofore prevented the utilization of this preparation for the induction of general anesthesia in man.

Sodium iso-amyl-ethyl barbiturate may, however, be prepared in a pure, anhydrous form, and if sealed in a glass vessel for protection against moisture and carbon dioxide is perfectly stable even when subjected to severe durability tests. The variations in anesthetic value previously noted appear to have been due in part to impurities, in part to slight variations in the pH value and in part to hydrolysis and decarboxylation on standing in solution.

Highly purified preparations of the sodium salt adjusted so that their 10% solutions in pure distilled water show pH values between 9.5 and 9.8 by the electrometric method, using the Bailey hydrogen electrode, give a maximum anesthetic effect with a minimum degree of toxicity when injected slowly by the intravenous route in dogs. A lowering of the alkalinity to the point at which a 10% solution shows pH values of 9.2 to 9.3, accompanied by the development of a slight opalescence or cloudiness due to commencing separation of traces of the barbituric acid compound, causes a striking loss in anesthetic value and a marked increase in toxicity. Such solutions even when slowly injected may cause Cheyne-Stokes breathing and other toxic effects on the respiratory center.

Since the sodium salt of iso-amyl-ethyl barbituric acid is almost entirely converted into the relatively water insoluble barbituric acid at a point considerably above that at which the water phase of the blood is supposedly buffered, pH 7.3 to 7.4, it might be anticipated that the injection of the 10% solution of the salt into the blood stream might easily result in sufficient precipitation to cause respiratory and other disturbances similar to those resulting from the injection of the already opalescent or slightly precipitated solution. There is, however, no trace of any such effect provided the solution is perfect and a given rate of injection is not exceeded, but even a perfectly prepared solution, if injected too rapidly, will affect the respiratory center. These results make it appear highly probable that the affinity of the cell surfaces and other lipoidal components of the blood for the released barbituric acid molecules is such that provided a given rate of injection is not exceeded, the accumulation of the product in the water phase of the blood does not reach the point of incipient precipitation with the accompanying toxic effect on the respiratory center.

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[1] Swanson, Edward E., and Page, Irvine H., *J. Pharm. and Exp. Ther.*, 1927, xxxi, 1.

40 to 50 mgm. per kilo of this preparation injected slowly by the intravenous route in a dog causes unconsciousness in 3 or 4 minutes and sufficiently deep anesthesia for surgical operation in 20 minutes. This anesthesia lasts for a period ranging from 1 to 3 hours depending on the dose employed. Dogs, when subjected to abdominal and other operations, have not exhibited any evidence of inadequate anesthesia, and the blood pressure has remained unchanged except in those cases in which severe hemorrhage has occurred.

Following anesthesia there is a somewhat prolonged period of post-operative sleep from which the dog may usually be aroused. However, it appeared desirable, before using this preparation extensively for the induction of general anesthesia in man, to find some means of antidoting or counteracting the post-anesthetic effects. This has been accomplished in dogs by injecting intramuscularly 1 mgm. ephedrine sulphate per kilo followed by 5 mgm. caffeine sodium benzoate per kilo. Dogs which have received such injections shortly after operation, when subsequently presented with food, will sniff the food, begin to eat and exhibit other signs of consciousness, whilst control dogs which have not received such injections may fail to react to the food.

This purified preparation of Sodium iso-amyl-ethyl barbiturate has given excellent results in the induction of general anesthesia in man in about 300 cases. The surgical anesthetic dose for man appears to be considerably smaller than that for the dog and ranges from 20 to 25 mgm. per kilo. There is no definite information available regarding the lethal dose of this preparation for man, but such meagre information as may be gathered from attempts at suicide, etc., makes it appear probable that the ratio between lethal and anesthetic dose is higher for man than for the dog. However, in the absence of any definite information regarding this point extreme caution has been observed in the conduct of these experiments and the dose employed has seldom exceeded 1.25 to 1.5 gm.

A dose of 10 to 15 mgm. per kilo has generally produced adequate anesthesia for satisfactory and unembarrassed manipulations in minor surgery. This dose has also sufficed for the control of convulsions in eclampsia, strychnine poisoning, epilepsy, tetanus and rabies, for periods of 6 to 10 hours.

20 to 25 mgm. per kilo have been employed to produce complete surgical anesthesia in cases in which other general or local anesthetics were contraindicated or inconvenient to administer, *e. g.*, mastoidectomy and operations on the face. However, for surgical an-

esthesia, wherever possible, this preparation has been used in conjunction with other general or local anesthetics.

The intravenous injection of from 12 to 20 mgm. of this preparation per kilo prior to the administration of ether or nitrous oxide and oxygen, has generally made it possible to reduce the amount of the supplementary anesthetic required to 20% or 25% of the quantity usually employed.

A similar dose used in conjunction with procain infiltration of the skin has permitted of extensive surgery of the abdomen with a greater degree of anesthesia and more muscular relaxation than when procain was used alone. It has seldom been found necessary in such cases to infiltrate the muscles and deep tissues with procain. The use of this combination seemed particularly advisable in those patients which were poor anesthetic risks.

The action exerted by Sodium iso-amyl-ethyl barbiturate when injected intravenously in diminishing the toxic and convulsive effects of local anesthetics, particularly procain, as recently reported by Loevenhart, Knoefel and Herwick,[2] and by Isenberger,[3] affords a further argument for the use of this preparation in combination with local anesthetics.

In surgery of the thyroid gland the technique of inducing anesthesia in the patient's room and without his knowledge, thus eliminating psychic stimulation of operating room and personnel, appeared highly desirable.

It is of the utmost importance that the preparation should be in perfect solution before being injected and that the rate of administration intravenously should not greatly exceed 1 cc. per minute. The patient is usually carried rapidly through the stages of anesthesia, so that excitement and laryngeal spasm are rarely observed. Sleep, as a rule, commences within 3 to 5 minutes and surgical anesthesia with complete relaxation of the muscles, and loss of the common reflexes except the pharyngeal, sphincters, etc., occurs within 15 to 20 minutes after starting the injection. The respirations are those of a patient in profound sleep, more often increased than decreased in rate, somewhat diminished in amplitude but regular. The blood pressure may decrease 15 to 30 mm. during the time of injection but rapidly regains its previous level.

Surgical anesthesia lasts for a period of 1 to 3 hours dependent in some measure on the dose employed. After maximal anesthetic doses the patients may sleep from 3 to 10 hours and occasionally longer. Nausea, retching and vomiting have not been observed in any of the cases. Frequently patients when coming out of sleep become restless before they are fully conscious. Morphine administered in the usual amount exerts a quieting effect.

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[2] Knoefel, P. K., Herwick, R. P., and Loevenhart, A. S., *J. Pharm. and Exp. Therap.*, 1928, xxxiii, 265.

[3] Isenberger, Robert M., *Proc. of the Staff Meetings of the Mayo Clinic*, 1928, iii, 40, 294.

In those cases in which maximal anesthetic doses have been employed, in which it is desired that the patient should awake somewhat sooner and fully alert mentally, 25 to 50 mgm. of ephedrine sulphate injected intramuscularly, followed by 0.65 to 1 gm. sodium caffeine benzoate, one-half to 2 hours after the operation, has exerted the desired effect.

Detailed papers covering the clinical, pharmacological and biophysical-chemical aspects of the case will shortly be published.

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A Consideration of the Pyridine Test for Chloroform.

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A method for the quantitative measurement of minute amounts of chloroform has been described by Cole.[1] An attempt was made to use this test to determine the amount of chloroform in sea water, in which small marine organisms were being anesthetized. Certain difficulties were encountered, many of which arose from the fact that the accuracy of the test was not known. This led to the study of the accuracy of the test discussed below.

A series of 105 tests were made upon a given concentration of chloroform in sea water. The test consists of mixing 2 cc. of 20% NaOH in distilled water, 1 cc. of pyridine and 1 cc. of the chloroform solution in a test tube and placing the latter in a water bath at 100° C., a red or pink color appearing in the mixture at this time. Precautions are taken to eliminate errors due to evaporation. When the chloroform is in sea water a precipitate forms; this is thrown down with a hand centrifuge. When distilled water is used no precipitate is formed. The tube is then cooled to about 18° C. and placed in a comparator box so arranged that several tubes can be arranged one behind the other, the color within the tubes being summated to give an intensity of color greater than that of any one of the tubes so arranged. The single tube containing the test solution is in this way compared with one or more standards and its chloroform content determined. The standards consist of tubes containing solutions of basic fuchsin in acidified 50% alcohol and are prepared according to Cole[1]; they are of various color intensities and represent 0.5, 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005 and 0.0001% of chloroform. From this series of tests it has been calculated that the mean deviation of a representative group of such measurements is not less than ±0.001.

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[1] Cole, William H., *J. Biol. Chem.*, 1926, lxxi, 173.

If a concentration of 0.03% chloroform can be measured to the fifth decimal place by this method, then the eye should be able to detect the difference in intensity when a 0.00005% color standard is placed behind a 0.03% standard in the comparator box mentioned above. Ten persons have been tested, therefore, as follows. The 0.5% standard is placed in the comparator box and each of the lower standards placed behind it. The person tested chooses the lowest standard which will just perceptibly increase the intensity of the 0.5 standard when placed behind the latter. In the same way, that standard is chosen which will just increase the intensity of the 0.1% color standard when placed behind it, and so on for each of the other standards. Standards were chosen in this way by each of the 10 persons; the results were averaged and are given in the table below.

Standard (A)	0.5	0.1	0.05	0.01	0.005	0.001	0.0005	0.0001
Average—10 persons (B)	0.17	0.03	0.007	0.0007	0.0002	0.0001	0.0001	0.0001
B/A	0.34	0.30	0.14	0.07	0.04	0.10	0.20	1.00

The ratio B/A is the ratio of a color standard which will just perceptibly increase the intensity of another color standard divided by the color standard the intensity of which is thus increased. This ratio is taken as a measure of the sensitivity of the eye to the particular intensity of color, the sensitivity increasing as the ratio decreases. It is to be noted that the sensitivity is greatest at about the 0.005% standard and decreases as the color standards increase and decrease. From the table it is evident that a 0.03% chloroform solution can be measured with an accuracy no greater than that implied by a figure in the third decimal place.

Cole[2] uses the pyridine test to determine the percentage of chloroform in extracts of the brains of rats killed by the inhalation of chloroform vapor. Knowing the volume of the extract and the weight of the brain, he calculates the quantity of anesthetic taken up per mgm. of brain and subsequently yielded to the extracting fluid. These results are carried to the fifth decimal place. From these figures it is concluded that the amount of chloroform present in the brains of rats at the time of death varies directly with the dosage. From the above table it is evident that the accuracy of the test does not justify the expression of such measurements to the fifth place. Furthermore, if one assumes that these figures are means of representative groups, each having a mean deviation of ±0.001 and the individual measurements of each falling within ±0.004, they should differ by at least 0.008 to be significant. As the dosage of chloroform increases, however, from 0.2 to 5.0 cc. per liter of air, only twice does the amount of chloroform per mgm. of brain tissue increase by as much as 0.008. From this it must be concluded that the measurements referred to do not show sufficient difference to justify their use as a basis for prediction.

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[2] Cole, William H., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 340.